INFLUENCE OF LIGHT EXPOSURE ON THE MELANOPSIN DRIVEN PUPIL RESPONSE AND CIRCADIAN RHYTHM

By

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THESIS

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DEDICATION

I dedicate this thesis to my Heavenly Father who has blessed me beyond all measure. I know that all good things come from Him.

To the love of my life, Megan:

The past four years have been the beginning of a wonderful, lifelong journey. Everything that I am and all that I am striving to be is for you and only because of you. May we always continue to support each other, grow, and never settle for anything less than our best.

To my parents and brother:

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An Abstract of a Thesis

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Abstract

Purpose:

Exposure to increasing amounts of artificial light during the night may contribute to the high prevalence of reported sleep dysfunction. Release of the sleep hormone melatonin is mediated by the intrinsically photosensitive retinal ganglion cells (ipRGCs). ipRGCs signal environmental light, with pathways to the midbrain to control pupil size and circadian rhythm. Evidence suggests that light exposure plays a role in refractive error development. This study sought to investigate whether melatonin level and sleep quality can be modulated by decreasing nighttime input to the ipRGCs. Another goal was to investigate links between light exposure, ipRGCs, refractive error, and sleep.

Methods:

Experiment 1: Fifty subjects, aged 17-40, participated (19 emmetropes and 31 myopes). A subset of subjects (n = 24) wore an Actiwatch Spectrum for one week. The Pittsburgh Sleep Quality Index (PSQI) was administered, and saliva samples were collected for melatonin analysis. The post illumination pupil response (PIPR) to 1 second (s) and 5s long and short wavelength stimuli was measured. Pupil metrics included the 6s and 30s PIPR and early and late area under the curve.

Experiment 2: Subjects (ages 17-42, n=21) wore short wavelength-blocking glasses prior to bedtime for two weeks. The ipRGC-mediated post illumination pupil response (PIPR) was measured before and after the experimental period. Stimulation was presented with a Ganzfeld stimulator, including 1 second (s) and 5 s long and short wavelength light, and the pupil was imaged with an infrared camera. Pupil diameter was

measured before, during and for 60 s following stimulation, and the 6 s and 30 s PIPR and area under the curve (AUC) following light offset were determined. Subjects wore an Actigraph device for objective measurements of activity, light exposure, and sleep. Saliva samples were collected to assess melatonin content. The Pittsburgh Sleep Quality Index (PSQI) was administered to assess subjective sleep quality.

Results:

Experiment 1: Subjects spent 104.8 ± 46.6 minutes outdoors per day over the previous week. Morning melatonin concentration (6.9 ± 3.5 pg/mL) was significantly associated with time outdoors and objectively measured light exposure ($P = 0.0099$ and 0.0016, respectively). Pupil metrics were not significantly associated with light exposure or refractive error. PSQI scores indicated good sleep quality for emmetropes (score 4.2 ± 2.3) and poor sleep quality for myopes (5.6 ± 2.2, $P = 0.036$).

Experiment 2: Subjects wore the blue-blocking glasses 3:57 ± 1:03 hours each night. After the experimental period, the pupil showed a slower redilation phase, resulting in a significantly increased 30 s PIPR to 1 s short wavelength light, and decreased AUC for 1 s and 5 s short wavelength light, when measured at the same time of day as baseline. Nighttime melatonin increased from 16.1 ± 7.5 pg/mL to 25.5 ± 10.7 pg/mL ($p < 0.01$). Objectively measured sleep duration increased 24 minutes, from 408.7 ± 44.9 to 431.5 ± 42.9 minutes ($p < 0.001$). Mean PSQI score improved from 5.6 ± 2.9 to 3.0 ± 2.2.

Conclusions:

The use of short wavelength-blocking glasses at night increased subjectively measured sleep quality and objectively measured melatonin levels and sleep duration, presumably as a result of decreased nighttime stimulation of ipRGCs. Alterations in the ipRGC-driven pupil response suggest shift in circadian phase. Results suggest that minimizing short wavelength light following sunset may help in regulating sleep patterns. Additionally, morning melatonin levels were influenced by light exposure and time outdoors. No differences in melatonin or the ipRGC-driven pupil response were observed between refractive error groups, although myopes exhibited poor sleep quality compared to emmetropes. Findings suggest that a complex relationship between light exposure, ipRGCs, refractive error, and sleep exists.

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Introduction

Background

Traditionally, rod and cone photoreceptors were believed to be the only photosensitive cells in the human retina. The existence of a third, non-traditional photoreceptor was first considered in 1923 when Keeler et al. observed a functional pupillary light reflex (PLR) in mice lacking the majority of their rod and cone photoreceptors.[1, 2] Keeler et al. then suspected the presence of a third photoreceptor in the retina; however, the maintained PLR could have persisted from a small number of functional rods and cones. In the 1990s, Russell Foster found a robust PLR, adaptable circadian rhythm to shifting light cycles, and suppression of melatonin release with light stimuli in mice devoid of traditional photoreceptors through genetic engineering.[3, 4] Further supporting the existence of a third photoreceptor, enucleated mice had previously been shown to lack PLR and circadian rhythm regulation.[5] More recently, normal pupil responses were found in subjects that were blind due to extensive outer retinal disease, begging the question as to whether or not another photoreceptor existed elsewhere in the retina.[6]

In 1998, Provencio et al. discovered a novel opsin-like photopigment in frog skin melanophores capable of responding to light which was dubbed melanopsin (opn4).[7] Remarkably, this opsin was found to be located in distinct retinal ganglion cells of the inner retina of mice and humans as well as the iris and deep brain nuclei.[7-9] However, proof of these retinal ganglion cells' ability to intrinsically detect light was still missing. Seeking to ascertain definitive proof of these new photoreceptors, Berson et al. completed a series of studies to isolate light activation of retinal ganglion cells from photoreceptors. Using a pharmacological cocktail, Berson et al. eliminated rod and cone signaling and

found that the retina was still responsive to light.[10] Berson et al. then mechanically isolated retinal ganglion cells and found the cells to still be responsive to light.[10] Immunochemistry validated the existence of melanopsin in these SCN-projecting ganglion cells.[11] Neither the absence of traditional photoreceptors nor of melanopsin completely abolishes the PLR.[12, 13]

Location and Number of ipRGCs

Melanopsin-expressing ganglion cells, now commonly referred to as intrinsically photosensitive retinal ganglion cells (ipRGCs), are unique in many ways, including their location in the retina. While traditional photoreceptors are located in the outer retina, ipRGCs are located in the inner retina and in a layer not entirely devoted to photosensitivity. Traditional photoreceptors also vastly outnumber ipRGCs. The human retina contains approximately 4.5 million cone photoreceptors and 90 million rod photoreceptors.[14] In comparison, ipRGCs comprise only 0.2% of total retinal ganglion cells in the macaque monkey estimating to be as few as 3,000 ipRGCs per eye.[15] The ganglion cell layer in mice is comprised of approximately 1% ipRGCs compared to humans 0.2%.[11, 15]

Projections

Mammalian models have demonstrated that ipRGC projections to brain centers are monosynaptic. Traditional retinal ganglion cells project to the lateral geniculate nucleus (LGN), olivary pretectal nucleus (OPN), and the superior colliculus (SC) to contribute to image formation, pupil constriction, and eye movements respectively.

Retrograde labeling has primarily been used for the determination of ipRGC axonal projections. Interestingly, these projections were traced to not just the traditional photoreceptor projection sites, but a wide array of other brain structures as well. ipRGCs project to the intergeniculate leaflet for the regulation of photoentrainment by the circadian system[16], the OPN for PLR control[11, 17], the ventrolateral preoptic nucleus (VLPO) for sleep regulation[18], and the SC.[15, 16] Also, stimulation of ipRGCs through light may have a direct affect on mood due to projections to the medical amygdala, lateral habenula, and subparaventricular zone.[19] Regulation of circadian rhythm is partially accomplished through a subset of ipRGC axons traveling to the SCN, which is the site of the mammalian master circadian clock.[20-22] ipRGCs have been shown to project to over a dozen distinct brain regions.[7, 10, 11, 15, 16, 20, 23] ipRGC functional LGN activity has been likely confirmed by the discrimination of high-irradiance lights from a dark background in mice lacking traditional photoreceptors through melanopsin stimulation with high-irradiance short wavelength light.[24] In the absence of traditional photoreceptors, circadian photoentrainment is still present.[3]

Intrinsic Pathway

Melanopsin-expressing ganglion cells are subject to both intrinsic melanopsin-driven stimulation, and indirect, extrinsic rod/cone-driven stimulation. Certain subtypes of ipRGCs (specifically M1) are driven primarily by their own melanopsin-mediated intrinsic stimulation, while other subtypes are stimulated more so through extrinsic stimulation.[25] Intrinsically, melanopsin is stimulated directly by short wavelength light with a peak sensitivity of 482 nm.[10, 15]

Extrinsic (synaptic) Pathway

As revealed through electron microscopy, rods and cones provide synaptic input to ipRGCs in the inner plexiform layer through bipolar (excitatory) and amacrine (inhibitory) cells.[15, 26-32] Non-M1 ipRGC subtypes are primarily stimulated extrinsically through cones and, to a lesser extent, rods.[25, 33] Through the combination of the intrinsic melanopsin stimulated pathway with the extrinsic synaptic pathway, ipRGCs can detect light intensities spanning a minimum of 9 orders of magnitude.[15] Projecting to the LGN, primate ipRGCs have a spatially overlapping, color-opponent (L+M-cone)-ON and (S-cone)-OFF receptive field structure.[15] The extrinsic ipRGC pathway allows for greater sensitivity, shorter latency, and stronger depolarization than is possible from the intrinsic pathway alone.

ipRGCs have been shown to express receptors for GABA, glycine, and glutamate which contribute to synaptic input.[28, 29] Dopaminergic amacrine cells provide modulatory input to ipRGCs,[34, 35] and also receive input from ipRGCs.[36]

Melanopsin

Unlike the photopigments of conventional photoreceptors, melanopsin is less dense, yet is diffusely located in the soma, dendrites, and proximal portion of the axons of ipRGCs.[23] Membrane density of melanopsin is approximately 10^4 fold lower compared to the membrane density of rod and cone photopigments.[23] Melanopsin expressing retinal ganglion cells are structurally similar to invertebrate rhabdomeric photoreceptors which depolarize in response to light, as opposed to rods and cones which hyperpolarize in

response to light.[10, 37] Expression of melanopsin in mice exhibits diurnal variation with increased expression at the end of the light phase and decreased expression at the end of the dark phase.[38]

OPN4 is the gene for melanopsin, and has been found in many mammalian species including humans, primates, and mice.[8] In melanopsin knock out mice, retinal ganglion cells retrogradely labeled from the SCN were unable to respond directly to light.[13] Additionally, in the absence of OPN4, mice showed diminished pupillary constriction and photoentrainment.[13, 39-42] OPN4 gene expression in cells which were normally insensitive to light resulted in robust responses to light, confirming that the OPN4 gene was capable of producing a photopigment.[43-45]

Evidence exists suggesting that melanopsin is regenerated intracellularly in all ipRGC subtypes though a bistable mechanism using long wavelength visible light.[46, 47] This is unique as it allows ipRGCs to not depend on an exogenous supply of chromophore, as rods and cones do, but rather regenerate from photoconversion.

ipRGC Subtypes

Although much is known about conventional retinal ganglion cells, the research on ipRGC subtypes, central projections, morphology, and functions is still in its infancy. Five ipRGC subtypes (M1-M5) have successfully been identified using transgenic mouse models.[25, 48] Identification has been made clear mostly by dendritic stratifications and aided by soma size. First visualized in the macaque monkey, ipRGC dendritic fields form a photoreceptive net that is concentrated parafoveally.[15] Early research suggests that ipRGC subtypes and pathways are conserved across different species.[49] In humans, two

subtypes of ipRGCs have been clearly identified[15, 50, 51] with some evidence now of four subtypes.[52]

Most projections to the SCN are accomplished by M1 axons while M2 axons provide mostly axons destined for the olivary pretectal nucleus.[16, 53] However, M1 axons also project to the OPN.[25, 54] Approximately 80% of M1 cells project to the SCN in rodents.[54] Likewise, approximately 80% of M1 and M2 cells project to the OPN with M1 axons chiefly projecting to the OPN shell while M2 axons largely projecting to the OPN core.[54] Dendritic stratifications are similar for M1 and M2.[32]

M1 cells are the most numerous and largest in size and were initially thought to be the only ipRGC. M1 cells are sparsely branched and have long dendrites, which differentiate in the OFF sublamina (outer IPL).[9-11]

M2 dendrites stratify in the ON sublamina (inner IPL) and are smaller in size, but have more complex dendritic arborization than M1 dendrites.[54-56] M1 and M2 subtypes comprise between 74-90% of ipRGC subtypes in the mammalian retina and their dendritic arbors exhibit much overlap.[55, 57] Compared to M2 cells, M1 cells demonstrate a larger membrane depolarization with roughly 10-fold higher sensitivity and also contribute the PLR more than M2 cells.[26, 57, 58] M1 and M2 cells express light contrarily with M1 primarily utilizing the intrinsic opn4 melanopsin pathway and M2 relying predominantly on synaptic stimulation from the outer retina.[57]

M3 cells are morphologically unique and distinguishable from other subtypes by their dendritic bistratification into both inner and outer IPL sublamina.[33, 57] M3 cells may be predominantly destined for non-image forming roles as their dendrites are absent in

some areas of the retina and complete retinal coverage is necessary for image forming functions.[55, 57] Projection destinations of the M3 axons are not yet known.

M4 and M5 subtypes were more difficult to identify due to inability to immunostain for melanopsin in these cells and have only been identified in mice. M4 and M5 cells do express marker proteins and possess the ability to depolarize without synaptic input and thus have an intrinsic, although weak, response.[59] M4 cells are morphologically differentiated by having the largest soma of all subtypes while M5 cells are described as "bushy", which describes their dense, extensively branched dendritic arborization.[59] Both M4 and M5 dendrites stratify in the ON sublamina (inner IPL).[59] M2, M4, and M5 cells all project to the dorsal LGN, which suggests image-forming functions for these subtypes.[59]

Estimations of relative population proportions between subtypes have proven to be challenging with M1 cells varying between 22-68%, M2 cells varying between 40-53%, and M3 cells varying between 7-26%.[54, 60]

Phototransduction

While classic photoreceptors signal with graded membrane voltages, ipRGCs signal using action potentials with higher amplitude per photon than rods and cones.[23] Stimulation of melanopsin occurs with >13 log quanta/cm^2/s of short-wavelength (blue) light.[58] Compared to rods and cones, ipRGCs contain a relatively sparse photopigment density and are somewhat inefficient at light absorption, thus requiring a higher irradiance for activation.[23] ipRGC kinetics are more sluggish in onset, slower to reach peak firing rate, and more gradual in termination than traditional photoreceptors. ipRGCs

are exceptionally tonic allowing continuous signaling for 10 hours.[10, 23, 61] Repolarization after light offset is significantly slower than in rods and cones with persistent spiking. The sluggish response kinetics indicate that that ipRGCs may be better suited for the integration of overall irradiance and ambient light levels over longer periods of time than traditional photoreceptors.[23] Findings from primates suggests ipRGCs to have unmyelinated axons in the retinohypothalamic tract, which is consistent with their slow conduction velocities.[62]

Signaling following stimulation offset lasts approximately 100x longer than cones and 20x than rods.[23] While the intrinsic response is sluggish, the extrinsic response is rapid and transient.[29] Unlike traditional photoreceptors, the intrinsic pathway of ipRGCs integrates photons over many seconds.[23] The "photoreceptive net," as described by Provencio et al., is composed of melanopsin containing dendrites which can also respond directly to light stimulation.[8, 9]

Photoreceptors are generally classified as either rhabdomeric or ciliary. Rhabdomeric photoreceptors are found in invertebrates while ciliary photoreceptors are found in rods and cones of vertebrates. Differences between the two classes lie in the photopigment molecule structure as well as the phototransduction cascade. Interestingly, ipRGCs do not fit strictly in either category as 11-cis-retinal is the absorbing molecule (similar to ciliary opsins), but uses a phototransduction cascade similar to the cascade for rhabdomeric opsins.[63]

Non-Image Forming Roles of ipRGCs

ipRGCs are responsible for many non-image forming functions, including circadian rhythm photoentrainment and pupil size control.[11, 64] ipRGC input to the SCN, which utilizes environmental irradiance to photoentrain the biological clock to solar day, is achieved predominately from M1 axons.[26, 54, 65]

Image Forming Roles of ipRGCs

Some evidence exists that ipRGCs may play a role in visual perception, specifically motion detection.[15, 59, 66] Given that conventional retinal ganglion cells and bipolar cells play a large role in the perception of brightness, it is conceivable that ipRGCs may also play a significant role in brightness perception, but this is not yet fully understood.[67] ipRGCs also may be involved with pattern vision,[59] color vision,[68] and contrast sensitivity. [69]

Development

ipRGCs develop much earlier than traditional photoreceptors. There is evidence that ipRGCs are capable of phototransduction in newborn mice when rods and cones are not yet formed.[70, 71] In fact, melanopsin is expressed before birth in mammals.[72] A potential explanation for such early development could be for a defense mechanism known as “negative phototaxis”, which is the response of head turning from bright light, occurring in mice as young as 6 days old.[73, 74] Evidence also exists suggesting that M4 cells partially regulate vascular development and ganglion cell layer neuron reduction.[75]

Post-Illumination Pupil Response

Melanopsin-expressing retinal ganglion cells partially regulate the pupillary light reflex.[58, 76] Neither the absence of traditional photoreceptors nor of melanopsin-expressing ganglion cells completely abolishes the PLR,[12, 13] but absence of melanopsin significantly attenuates the PLR.[26, 65] The most significant contribution of ipRGCs to the PLR is the post-illumination pupil response (PIPR), which is a sustained constriction of the pupil occurring after short-wavelength light offset. PIPR is suggested to be a direct measure of ipRGC function in humans and animals.[58, 76, 77] The PLR is also the only measurable, non-invasive, physiological response, which originates from all three photoreceptor types in the retina. Non-invasive pupillometry techniques allow for objective measurement of inner (ipRGCs) and outer (rods/cones) retina function using both long and short wavelength stimuli of various irradiances.[76-82] Measurable and analyzable components include latency to constriction, maximum constriction, PIPR amplitude, transient pupil response, and redilation constant.[76, 77, 79, 81-87]

Contributions of specific photoreceptors to the PLR and PIPR are dependent on the light stimulus parameters. Being highly sensitive, rods dominate the PLR under conditions of dark adaption, and cone contributions occur with increased light intensity or light adaption. Initial pupil constriction is mediated by the rods and cones of the outer retina.[26, 76] For stimuli under 10 seconds in duration, the pupil response is dominated by rods with a small contribution by cones and an increasing contribution by ipRGCs with longer stimuli durations.[76] The ipRGC contribution to PIPR is observed as a sustained pupil constriction after the offset of a high-irradiance, short-wavelength light stimulus.[58, 76, 88] PIPR is commonly quantified as a percentage of baseline pupil diameter at a

specified time point after light offset, often 6 seconds.[58, 77, 78] Other parameters of the PIPR include the net PIPR, which is the difference in the amplitude of the response to produced using long versus short wavelength stimuli,[79, 84] and the early or late area under the curve (AUC).[87] PIPR displays 24-hour circadian modulation, which is not observed in predominantly cone-driven pupil responses.[78, 89]

There are conflicting reports as to whether PIPR varies with age, with two studies indicating PIPR does not vary with age,[83, 90] and one studying suggesting PIPR may vary with age.[86] However, as the intraocular lens ages, transmission of blue light to the retina is limited giving the possibility of PIPR age variation in the absence of ipRGC light sensitivity adaptation.[86]

ipRGCs and Retinal and Optic Nerve Disease

PIPR can be affected in retinal and optic nerve disease. Glaucoma can reduce ipRGC driven PIPR.[80, 83, 84] The severity of glaucomatous optic neuropathy is inversely related to the PIPR, with the more advanced stages resulting in a smaller PIPR.[84] Neuronal death is non-selective in glaucoma and affects both ipRGCs and conventional ganglion cells. In subjects with glaucoma, PIPR is affected before circadian rhythm where mild/moderate cases only affect PIPR, but advanced cases may affect not only PIPR, but result in increased daytime sleepiness due to the lack of melatonin suppression by ipRGCs.[91] In cases of unilateral glaucoma, the glaucomatous eye may have a reduced PIPR while the unaffected eye retains normal pupillary responses.[92] PIPR has also been correlated with visual field defects with advanced glaucoma.[84]

Reduced ipRGC function has been observed in patients with diabetic retinopathy.[93] Remarkably, PIPR can be reduced in diabetic subjects with no retinopathy.[81] This suggests that PIPR evaluation could be a useful non-invasive method for determining inner neuroretinal changes in diabetic patients showing no retinopathy through routine ophthalmological examination. Reduced transient pupillary constriction measured in diabetics could indicate outer retinal damage as well.[81]

Interestingly, melanopsin-expressing ganglion cells can be more resistant to insult than other retinal ganglion cells. Neuronal death is selective for classic ganglion cells in hereditary optic neuropathies, such as Leber's hereditary optic neuropathy and dominant optic atrophy, while ipRGCs are more resilient to insult.[94, 95] Similarly, in cases of ocular hypertension and axotomy, non-melanopsin-expressing ganglion cells are typically damaged before ipRGCs.[96, 97] In retinitis pigmentosa, both extrinsic and intrinsic pathways of ipRGCs are lost with disease progression.[98] In advanced stages of retinitis pigmentosa, ipRGC density and dendritic arborization decrease.[99] Interestingly, melanopsin mediated PIPR is preserved in some cases of retinitis pigmentosa but extremely low in other cases.[98, 100, 101]

In macular degeneration, anatomical and functional disruptions primarily involve the paracentral retina, where ipRGCs are most concentrated.[15, 102] In advanced stages, AMD results in damage to both the outer and the inner retina, including retinal ganglion cells.[103, 104] Therefore it is reasonable to postulate that ipRGCs may be affected in AMD. While some retinal ganglion cells are lost simply due to the aging process, neovascular AMD has demonstrated a 50% loss of retinal ganglion cells.[104] Both early dry AMD and neovascular AMD may display inner retinal dysfunction indicated through PIPR.[105]

Patients with AMD have also been shown to have higher levels of melatonin, presumably due to decreased ipRGCs and subsequent alterations in melatonin release, which may lead to severe circadian rhythm disturbance.[106] This may predispose AMD patients to depression and cognitive impairment, along with mood and sleep disorders.

The PIPR as a marker of ipRGC function may prove useful in the objective evaluation of progressing retinal or optic nerve diseases due to reduced function of ganglion cells, particularly melanopsin-expressing ganglion cells. Early evidence exists that a clinical protocol could be developed to access rod, cone, and ipRGC contributions to PIPR in cases of outer retinal disease.[82, 98] Furthermore, the PIPR is more sensitive than standard electroretinography in advanced stages of disease for detecting residual photoreceptor activity.[100, 107] Isolation of outer and inner retinal function may be accomplished through adjustment of stimulus size and retinal irradiance, with larger stimulus sizes being more sensitive to inner retina function, smaller stimulus sizes being more sensitive to outer retina function, and retinal irradiances below melanopsin threshold isolating outer retina function.[77, 79, 108, 109]

Circadian Rhythm

The SCN of the hypothalamus controls the daily rhythms of physiology and behavior in mammals. This biological clock controls sleep/wake cycles, gene expression, and body temperature, and is roughly tuned to a 24-hour day, but not precisely. Therefore, exogenous cues are used to adjust and tune the biological clock, the most potent of which is light. The synchronization to light/dark cycles is known as

photoentrainment. ipRGCs are the primary photoreceptors involved, as knocking out melanopsin abolishes photoentrainment in mice.[26, 65, 110]

The contribution of ipRGCs to circadian rhythm regulation occurs through modulation of melatonin release into the bloodstream by the pineal gland. ipRGCs project to the SCN which signals along a pathway or circuit to the paraventricular nucleus of the hypothalamus, intermediolateral nucleus of the spinal cord, superior cervical ganglion, and finally to the pineal gland.[111] Stimulation of ipRGCs suppresses the release of melatonin from the pineal gland, thus resulting in melatonin release only occurring during subjective night.[112-114] PIPR is a non-invasive marker of circadian rhythm because ipRGC circadian response is synchronized with melatonin onset while outer retinal inputs are not.[78]

Evidence for the role of ipRGCs in the circadian system is supported by findings that humans, as well as mice, who were blind due to rod and cone photoreceptor degeneration could still maintain normal circadian function.[4, 6, 115] Conversely, melanopsin null mice maintain normal circadian rhythm photoentrainment to 12h:12h light-dark cycles, but with mitigated short-wavelength induced circadian rhythm shifts.[39] Some, but not all, blind persons suffer from chronic sleep disorders.[116, 117] Such evidence suggests that ipRGC dysfunction may contribute to circadian misalignment and human disease. Evidence also suggests that light exposure during evening hours can lead to chronodisruption, or impaired physiological, behavioral and biochemical rhythms.[118]

ipRGCs predominately, if not exclusively, mediate sleep-wake regulation through activation of neurons in the VLPO, while rods and cones were found to have no such role.[119] Melanopsin also mediates sleep homeostasis.[120] Perhaps the most notable

physiological marker of circadian phase is melatonin whose nocturnal secretion can be suppressed by short-wavelength light.[121] The extent and direction of circadian shifts induced by light is dependent of the spectral composition, time of day, irradiance, and duration.[122] A short-wavelength shifted spectrum results in augmented responses to monochromatic blue light as compared to the effect of the longer wavelength green and red light for melatonin suppression, sleep regulation, PLR, circadian clock gene expression, alertness, mood, emotional processing, core body temperature, and cognition.[58, 113, 114, 123-132]

Melatonin

Stimulation of ipRGCs by high-intensity, short-wavelength lights results in a cascade of events eventually leading to the suppression of melatonin synthesis and release from the pineal gland.

Melatonin is primarily produced at nighttime in dim illumination.[133, 134] It is well established that melatonin levels decrease with age.[135] Melatonin functions through G-protein coupled receptors located in the peripheral and central nervous systems, but primarily in the SCN.[136-141] Melatonin is known to regulate sleep/wake cycles as well as core body temperature.[142, 143] Lack of melatonin at nighttime impairs sleep quality.[144] Also, bright light in the evening hours suppress melatonin production and release.[121]

Abnormalities in circadian rhythm have been linked to a number of conditions, including Alzheimer's,[145] dementia,[146] bipolar disorder,[147] diabetes,[148] hypertension,[149] cancer,[150] and cardiovascular disease,[151, 152] and more.[153]

Sleep

The National Sleep Foundation estimates that adolescents routinely use cell phones (72%) and computers (60%) in the hour before sleep.[154] Increased exposure to back-lit screens is strongly linked to poor sleep in adolescents.[155] Only 50% of adolescents report getting a good night's sleep during the weeknights, and up to 25% report sleeping less than 6 hours on school nights.[156]

Zeitgebers are inputs to human circadian rhythm regulation that allow humans to synchronize to the 24 hour day, the strongest of which is light.[157] It is known that bright evening light suppresses endogenous melatonin release.[158-160] Doses of light as low as 100 lux have been found to suppress melatonin, resulting in increased alertness.[161, 162] In adult subjects, one hour of electronic tablet use with dim illumination (40 lux) did not decrease melatonin levels. However, when the exposure was increased to two hours, melatonin levels were suppressed.[163] Wood et al., found that wearing short wavelength blocking amber tinted glasses for two hours while exposed to a bright screen can counter the melatonin suppression.[163]

An increasingly common method of reducing short-wavelength exposure in evening hours is through an application called f.lux. F.lux has been shown to alter the peak spectral luminance of computer monitors from 453 nm to 597 nm, decreasing stimulation of ipRGCs.[164] The same study discovered that the use of f.lux for one evening hour was not found to significantly alter melatonin levels or alertness.[164] The researchers did not preclude that longer use of f.lux could result in a significant difference in melatonin levels or alertness. Other studies have shown that wearing blue-blocking

glasses at nighttime for two weeks improves sleep quality and overall mood, while also alleviating blue LED-induced nighttime melatonin suppression.[165, 166]

Other ipRGC Behavior Aspects

Irregular light exposure patterns causing sleep deprivation and circadian disruption can trigger mood and cognitive impairment.[167] In normal mice, an irregular light pattern has been shown to depress moods and impair learning; however, when mice lacking ipRGCs are subjected to an irregular light pattern, mood and learning remains unchanged.[168] This result suggests that ipRGCs play a significant role in mood and learning in mammals. A study revealed that light modulates responses to learned fear through ipRGC pathways in addition to cognition and anxiety.[169] It has also been shown that ipRGCs contribute to cognitive function and alertness in humans.[126, 170,171] Patients with gene variants of the melanopsin OPN4 gene are predisposed to seasonal affective disorder. [172] Additionally, stimulation of ipRGCs with blue light helps combat depression in humans and mice.[173, 174] The human circadian clock has been shown to be susceptible to phase shifts when exposed to blue light (440um-480um), which may improve alertness and mitigate seasonal affective disorder.[172, 175] There are also strong indications that ipRGC stimulation may exacerbate migraines through short-wavelength light stimulation as ipRGCs project to dura-sensitive neurons of the posterior thalamus.[176] ipRGC stimulation triggers light avoidance and photophobia in mice.[177, 178] Other biological functions of ipRGC stimulation include relaxation of blood vessels[179] and secretion of follicle-stimulating hormone in women.[180]

Dopamine and the ipRGCs

ipRGCs receive modulatory input from dopaminergic amacrine cells,[34, 35] and also have reciprocal signaling to dopaminergic amacrine cells.[36] Dopamine also regulates melanopsin mRNA expression in ipRGCs.[181] Animal models demonstrate that an increase in light exposure leads to increased retinal dopamine levels.[29] It is possible that the synaptic connections between ipRGCs and dopaminergic amacrine cells could explain the increase in retinal dopamine upon light exposure. Supporting this idea, light induced dopamine release is suppressed when melanopsin is knocked out.[182] Whether ipRGCs significantly increase the levels of retinal dopamine has not yet been clarified.[183]

Outdoor Light, Refractive Error, and ipRGCs

Myopia has quickly become a global pandemic, with some developed countries reaching a prevalence of over 90%.[184] Refractive error is influenced by both genetic and environmental factors.[185, 186] Exposure to outdoor light has been demonstrated to be protective in the development of myopia in children.[187, 188] Interestingly, myopia has been associated with poor sleep quality[189, 190] and lessened sleep duration.[190] Furthermore, evidence suggests that myopes may have higher concentrations of morning melatonin.[191] With the role of ipRGCs as environmental irradiance detectors[42] and the myriad of physiological contributions mentioned previously, including sleep and melatonin levels, questions exist as to whether ipRGCs may play a role in axial length and refractive error development.

Our Research

Short-wavelength light is known to have a significant impact on circadian rhythm through ipRGC stimulation and studies suggest circadian rhythm could be modulated through the manipulation of ipRGC input, thus influencing systemic melatonin levels. The ability to alter melatonin levels through the manipulation of ipRGC input could prove tremendously useful in the world of sleep disorders, nightshifts in the workforce, jet lag, and general circadian rhythm dysfunction.

Chapter two details a study that investigated if ipRGCs are responsible for the previously observed improvements in sleep metrics following the wear of blue blocking glasses in the evening hours. ipRGC activity was indirectly measured through the PIPR in an attempt to better understand its contributions to sleep metrics and systemic melatonin levels. We hypothesized that attenuating short wavelength light in the evening hours would result in an increase in nighttime melatonin and an improvement in sleep metrics. Additionally we expected a decrease in morning melatonin levels and potentially, an increase in PIPR.

Evidence suggests that eye growth may be influenced by light exposure and time outdoors, although the mechanisms behind the relationship are unknown. A potential factor could be retinal dopamine levels, which are influenced by ipRGCs. With myopia becoming a growing concern for both refractive reasons and the ocular health implications, determining underlying mechanisms for axial elongation and preventative measures is of growing importance. Investigation into potential relationships between light exposure, refractive error, and PIPR could contribute to better understanding environmental factors and mechanisms influencing eye growth and circadian rhythm.

Chapter three details a study that sought to examine the relationship between the ipRGC-driven pupil response, light exposure, and refractive error. We also examined downstream relationships between light exposure, sleep metrics, and melatonin in myopic and emmetropic individuals.

Chapter Two

The ipRGC-Driven Pupil Response with Light Exposure, Refractive Error and Sleep

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Abstract

Significance: We investigated links between the intrinsically photosensitive retinal ganglion cells, light exposure, refractive error, and sleep. Results showed that morning melatonin was associated with light exposure, with modest differences in sleep quality between myopes and emmetropes. Findings suggest a complex relationship between light exposure and these physiological processes.

Purpose: Intrinsically photosensitive retinal ganglion cells (ipRGCs) signal environmental light, with pathways to the midbrain to control pupil size and circadian rhythm. Evidence suggests that light exposure plays a role in refractive error development. Our goal was to investigate whether links exist between light exposure, ipRGCs, refractive error, and sleep.

Methods: Fifty subjects, aged 17-40, participated (19 emmetropes and 31 myopes). A subset of subjects (n = 24) wore an Actiwatch Spectrum for one week. The Pittsburgh Sleep Quality Index (PSQI) was administered, and saliva samples were collected for melatonin analysis. The post illumination pupil response (PIPR) to 1 second (s) and 5s long and short wavelength stimuli was measured. Pupil metrics included the 6s and 30s PIPR as well as early and late area under the curve.

Results: Subjects spent 104.8 ± 46.6 minutes outdoors per day over the previous week. Morning melatonin concentration (6.9 ± 3.5 pg/mL) was significantly associated with time outdoors and objectively measured light exposure (P = 0.0099 and 0.0016, respectively). Pupil metrics were not significantly associated with light exposure or refractive error. PSQI scores indicated good sleep quality for emmetropes (score 4.2 ±

2.3) and poor sleep quality for myopes (5.6 ± 2.2, P = 0.036).

Conclusions: We found that light exposure and time outdoors influenced morning melatonin concentration. No differences in melatonin or the ipRGC-driven pupil response were observed between refractive error groups, although myopes exhibited poor sleep quality compared to emmetropes. Findings suggest that a complex relationship exists between light exposure, ipRGCs, refractive error, and sleep.

Keywords: intrinsically photosensitive retinal ganglion cells; pupil; melanopsin; light exposure; melatonin; sleep; refractive error

2.1 Introduction

Myopia is an epidemic, reaching a prevalence of over 90% in some urbanized countries.[184] Myopia represents a large socioeconomic burden and poses a risk for associated ocular diseases including retinal detachment, neovascularization, and glaucoma.[192] Evidence suggests that refractive development and eye growth are regulated by a complex interaction between genetic and environmental factors.[185, 186] Time outdoors and light exposure have been shown to be protective of myopia in children.[187, 188]

Light exposure is associated with dopamine and melanopsin signaling. The intrinsically photosensitive retinal ganglion cells (ipRGCs), located in the inner retina, are directly stimulated by light through activation of the photopigment melanopsin, with a peak sensitivity around 482 nm.[8] IpRGCs account for less than 1% of the ganglion cell population, with widespread dendritic coverage encompassing the entire retina (except at the fovea),[32, 55] and are responsible for non-image forming functions including circadian rhythm entrainment and pupil size control.[11] Axons project to the olivary pretectal nucleus for control of pupil size,[193] and to the suprachiasmatic nucleus in a pathway ultimately leading to the pineal gland to control the release of the sleep hormone melatonin. The ipRGCs are considered environmental irradiance detectors, and have been implicated in a myriad of physiological processes, including metabolism, mood, and sleep.[42, 127, 194]

In addition to direct stimulation, the ipRGCs receive extrinsic synaptic input through the rod and cone pathway, with connections to bipolar and amacrine cells.[29] Studies utilizing animal models have demonstrated that increased light exposure leads to

an increase in retinal dopamine,[195] which may, in part, be mediated by synaptic connections between ipRGCs and dopaminergic amacrine cells.[196] Single cell recordings show that extrinsically driven ipRGC responses are rapid and transient, while intrinsic ipRGC responses show a longer latency and sustained firing during stimulation, and continue to fire following stimulus offset.[15] In vivo ipRGC activity can be measured indirectly through the pupil response to short wavelength light.[58] Following short wavelength stimulation offset, the post illumination pupil response (PIPR) redilation dynamics have been shown correlate with the sustained firing pattern seen in single cell recordings,[58] and are an indicator of ipRGC activity.

The ipRGCs provide environmental light information to the suprachiasmatic nucleus to synchronize sleep/wake patterns.[41] Recent studies have found that myopia may contribute to decreased sleep quality.[189, 190] Jee et al., found an inverse relationship between sleep duration and myopia in Korean adolescents.[190] Similarly, another study reported that children with high myopia exhibited the poorest scores on the Pittsburgh Sleep Quality Index (PSQI), a sleep quality questionnaire.[189] The authors attributed the observed decreased sleep quality to high demands in school, distress over poor vision, or decreased ipRGC function in myopic eyes. Sleep, circadian rhythm, and ipRGC activity have also been shown to be affected in diseases, which affect the inner retina, such as glaucoma,[197] and in outer retinal diseases, including age related macular degeneration.[198] Another study reported a positive association between morning melatonin concentration and the magnitude of myopia, with myopes demonstrating up to three times greater melatonin concentration than non-myopes.[191] Therefore, it is possible that ipRGCs could also be affected with axial myopic eye growth, in which the retina is subject to structural

and functional changes.[199] A recent report found no effect of refractive error on ipRGC inputs to the pupil control pathway.[90] However, another investigation found a significant positive correlation between a more hyperopic refractive error and a slower rate of pupil redilation to 0.1 Hz flashing stimuli, which varied as a function of light exposure over the previous five days.[200] Differing results and current interest in light exposure and myopia warrant further investigation into potential relationships between light exposure, refractive error and pupil responses.

The goal of this study was to investigate relationship between the ipRGC-driven pupil response and light exposure to begin to understand the interaction between environmental factors and potential mechanisms influencing eye growth and circadian rhythm. We also examined whether downstream relationships existed between light exposure, sleep quality, and melatonin in myopic and emmetropic individuals.

2.2 Methods

Subjects, ages 17-40, were recruited for this study. All subjects provided informed consent after the purpose of the study and the risks were explained. The study was approved by the Committee for Protection of Human Subjects at the University of Houston and followed the tenets of the Declaration of Helsinki. Exclusion criteria included ocular disease and the use of melatonin or other pharmacological sleep aids.

Lab visits occurred between 9:00 am to 11:00 am to avoid potential effects of circadian variation.[78, 89] Visual acuity was measured with habitual correction, and an anterior eye exam using slit lamp biomicroscopy was performed to confirm angles were open and suitable for dilation. Acuity for all subjects was 20/25 or better and no subject

had ocular pathology, including cataracts or glaucoma. No subjects were taking prescription medications known to affect pupil size.

The experimental protocol is shown in Figure 2-1A. The Pittsburg Sleep Quality Index questionnaire (PSQI) was administered to measure sleep quality subjectively, and saliva samples were collected to measure melatonin concentration. Prior to collection, subjects were instructed not to eat, drink coffee, or brush their teeth within an hour of the lab visit. In addition, subjects were asked to abstain from alcohol and nicotine 12 hours prior to the lab visit. Approximately 1 ml of saliva was collected and immediately placed in a -20° C freezer for later analysis using a melatonin ELISA kit (Salimetrics, CA, USA). All samples were run in duplicate.

Objective Light Exposure Measurement

A subset of subjects (n = 24) wore an Actiwatch Spectrum (Philips Resipronics, OR, USA) for one week prior to the lab visit. The Actiwatch Spectrum is a non-invasive wrist-worn activity monitoring method that measures ambient light exposure and activity continuously at 32 Hz. This device has been actively used in both children and adults in various applications, including light exposure, activity, and sleep related studies.[201, 202] The light sensor in the Actiwatch Spectrum consists of color sensitive photodiodes to measure the illuminance of white light in units of lux (range 0.1 to 200,000 lux), and the irradiance of the blue (400-500 nm), green (500-600 nm), and red (600-700 nm) components in $\mu W/cm^2$. Each subject was asked to wear the device for 7 days, which was set to average over one minute epochs. Subjects were instructed not to remove the device for the entire measurement period, including during sleep. Days were excluded from the

analysis if the subject removed the device for more than 30 minutes, or if the light exposure dropped to zero for 30 minutes or more during daylight hours, indicating obstruction of the light sensor by clothing. An "off-wrist" sensor in the device and absence of missing data showed that all subjects were compliant with the requested wear regimen.

Actiwatch data were analyzed with the device software, Philips Actiware 6.0.8, as described previously.[202] Time outdoors was defined as mean minutes per day exposed to > 1000 lux. For subjects who did not wear the Actiwatch, a brief survey was administered to determine time outdoors over the previous week. Light exposure was analyzed in terms of natural log cumulative white (broad band) light (lux), as well as for the blue and red spectral output ($\mu W/cm^2$). To understand the integration time for potential relationships between melatonin and pupil metrics with light exposure, we calculated the cumulative light exposure binned into four intervals, 1 day, 3 days, 5 days, and 7 days. Sleep duration and efficiency were calculated for the week.

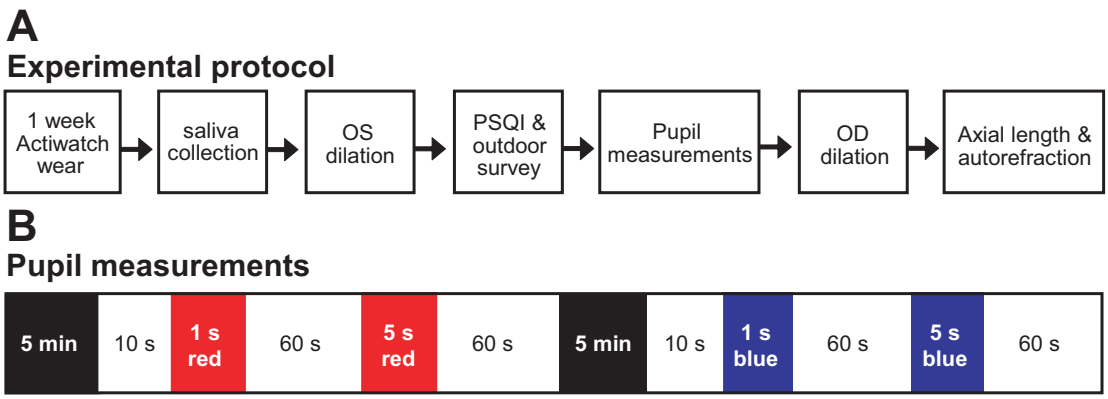

Figure 2-1. A) Experimental protocol B) Pupil stimulation protocol. Dark adaptation (5 m) was followed by 1 s and 5 s long wavelength stimulation, with 60 s pupil following the stimulus. The protocol was then carried out with short wavelength stimuli.

Pupillometry

Following saliva collection, the left eye was dilated with 2.5% phenylephrine and 1% tropicamide. Dilation of the left eye was employed in an effort to maintain consistent retinal illumination within and between subjects during stimulation. After a 20 minute dilation period, the ipRGC driven pupil response was measured via the post-illumination pupil response (PIPR) in the right eye, as described previously.[203] Briefly, a frame mounted binocular eye tracker with infrared illumination (ViewPoint EyeTracker, Arrington, AZ, USA) was used record the pupil size of the right eye at 60 Hz. The IR LED light source has a lambda max of 943 nm with a half-max width of 46 nm (Ocean Optics Spectrometer, FL, USA). Pupil size was calibrated for each subject by first capturing an image of a 5mm artificial pupil. Following calibration, subjects dark adapted for 5 minutes (< 1 lux), then placed their head in a chinrest with an LED-driven Ganzfeld system (Color Burst, Espion, Diagnosys LLC, MA, USA) centered in front of the left eye at 10 mm providing full field stimulation. Subjects viewed a red fixation point directed at the wall at 10 feet with the right eye during measurements.

Stimuli presented to the left eye consisted of long wavelength (red) and short wavelength (blue) narrowband 1 second (s) and 5 s pulses of light (Figure 2-1B). Previous studies have utilized stimulus durations of 4 ms to 30 s; here, two durations that were within the range of previously published studies were utilized.[204] While previous studies have concluded that a 1 s stimulus is sufficient to stimulate the melanopsin pathway, we also included the 5 s stimulation to understand if PIPR metrics would be different for longer stimulations. Long wavelength light was 651 nm with a half-max width of 25 nm (Spectroradiometer CS1W, Minolta), and corneal irradiance was 5.58 x

10^{13} photons/cm^2/s (Power Meter, Newport Corporation, CA, US). Short wavelength light was 456 nm (half-max width of 20 nm), with a corneal irradiance of 5.85 x 10^{13} photons/cm^2/s. After dark adaptation, the baseline pupil diameter was measured in the dark for 10 s. A long wavelength 1 s pulse was presented to the left eye, and the consensual pupil diameter was measured for 60 s. Then a long wavelength 5 s pulse was presented and the consensual pupil diameter was again measured for 60 s. The subject dark adapted for 5 minutes, and the protocol was repeated with short wavelength light. Previous evidence suggests that prior long-wavelength light exposure enhances short-wavelength induced pupil constriction.[205] Therefore, both long wavelength stimuli were presented, followed by a dark adaptation period, and then blue stimuli were presented, similar to previously published experiments.[89]

Following ipRGC measurements, the right eye was dilated. After 20 minutes, axial length (LenStar, Haag-Streit) and refractive error (WAM-5500, Grand-Seiko, Japan) were measured in both eyes. For axial length, three measurements were recorded and averaged for each eye, then both eyes were averaged together. For refraction, the spherical equivalent refractive error (SER) was calculated for the average of 5 measurements for each eye, and both eyes were averaged together. Subjects were grouped based on refractive error; the emmetropic group included SER from +1.25 to -0.5 D, and the myopic group included SER < -0.50. Emmetropic and myopic groups were age and sex matched.

Data Analysis

Results are presented as mean ± standard deviation. Primary outcomes were pupil metrics, sleep quality, and melatonin. Statistical analysis was performed in MedCalc (MedCalc Software, Ostend, Belgium). Normality was confirmed with the Kolmogorov-Smirnov test, and outliers were detected with a Tukey outlier filter. The Kolmogorov-Smirnov test showed that light exposure, pupil metrics and melatonin were normally distributed, so statistical analyses of these variables were performed with simple linear regression models and significance was determined based on the p value for slope less than 0.05. Refractive groups were compared with unpaired two tailed t-tests and Mann-Whitney tests for independent samples.

Pupil data were analyzed off line using a custom MATLAB program (MathWorks, Natick, MA). Blinks were identified as intervals of pupil-aspect ratio outside 6 SD of the mean pupil aspect ratio during stable fixation, or samples deemed poor quality by the instrument, and removed from the trace. Pupil metrics are defined in Table 2-1. The baseline pupil diameter was calculated by averaging pupil diameter during the 10 s pre-stimulus period. Normalized responses were determined by dividing pupil diameter by the baseline pupil diameter (expressed in percent). Peak constriction was calculated as the percentage of the maximally constricted pupil diameter to baseline pupil diameter. The 6 s and 30 s PIPR were calculated as the normalized pupil size averaged over 6-7 s and 30-31 s, respectively, after each stimulus offset. Early and late area under the curve (AUC) were computed for the recovery intervals 0 to 10 s and 10 to 30 s following stimulus offset.[87] The areas were computed as the trapezoidal approximation of the integral of 100% minus the interpolated percent pupil diameter (i.e the difference

between the pupil and baseline) for the respective intervals (unitless). The pupil metrics were analyzed in terms of SER, morning melatonin concentration, time outdoors, and natural log cumulative light exposure.

Table 2-1. Pupil metrics utilized to quantify photoreceptor contributions

Metric	Definition	Unit	Expected change
Baseline pupil diameter	Dark adapted 10 s pre-stimulus pupil diameter	mm, defined as 100%	
Peak constriction	Maximum pupil constriction	% of baseline pupil diameter	Smaller value indicates greater constriction
6 s PIPR	Mean pupil diameter 6-7 s after stimulus offset	% of baseline pupil diameter	Smaller value indicates greater ipRGC activity
30 s PIPR	Mean pupil diameter 30-31 s after stimulus offset	% of baseline pupil diameter	Smaller value indicates greater ipRGC activity
Early AUC	Integral of 100% minus the interpolated % pupil diameter, 0-10 s after stimulus offset	unitless	Larger value indicates greater ipRGC activity
Late AUC	Integral of 100% minus the interpolated % pupil diameter, 10-30 s after stimulus offset	unitless	Larger value indicates greater ipRGC activity

2.3 Results

Fifty subjects participated (24 female, 26 male), with a mean age of 26.9 ± 6.2 years (mean ± SD; range 17-40, Table 2-2). Mean cycloplegic SER was -2.5 ± 2.7 D (range -7.8 to +1.2 D) and mean axial length was 24.6 ± 1.2 mm (range 22.3 to 26.9 mm). Mean SER of the emmetropic group was +0.38 ± 0.48 D (+1.2 to -0.50 D, n = 19) and of the myopic group was -4.3 ± 1.6 D (-1.5 to -7.8 D, n = 31). Mean axial length of the emmetropic group was 23.6 ± 0.8 mm (22.3 to 25.3 mm) and of the myopic group was 25.3 ± 0.9 mm (23.2 to 26.9 mm).

Light exposure, sleep, and melatonin

For all subjects, time outdoors during daylight hours over the previous week was 104.8 ± 46.6 minutes per day (range 22 to 249 minutes). The subject who reported spending 249 minutes per day outdoors was identified as an outlier and not included in analyses. For the subset of subjects who wore the Actiwatch device (n = 24), natural log daily light exposure was 14.0 ± 0.66 lux (range 12.5 to 15.2 lux). For morning melatonin concentration, 43 subjects were included in the analysis; 7 subjects did not provide adequate saliva samples. Mean melatonin concentration, measured in the morning prior to pupil measurements, was 6.9 ± 3.5 pg/mL (range 0.84 to 17.8 pg/mL); two subjects with the highest values were identified as outliers and not included in regression analyses.

Increased time outdoors during the previous week was associated with higher morning melatonin concentration ($R^2 = 0.23$, $F = 11.6$, $df = 38$, $P = 0.0016$, Figure 2-2A). For the subset of subjects with objective light exposure data, cumulative white light exposure was binned into four time slices (1 day, 3 days, 5 days, and 7 days) with Bonferroni corrected significance level of $P \leq 0.013$. Morning melatonin concentration

was significantly associated with white light exposure over the previous 7 days, (R^2 = 0.29, F = 8.12, df = 20, P = 0.0099, Figure 2-2B), but not the previous 5 days (P = 0.02), 3 days (P = 0.05) or 1 day (P = 0.51). Morning melatonin concentration was also significantly associated with red and blue light exposure over the previous 7 days (Figure 2-3). However, red and blue light exposure were highly correlated with white light exposure (R^2 = 0.97, P < 0.0001 for both, Figure 2-4); therefore, red and blue were not considered independent variables. There were no significant refractive error group differences in time outdoors, light exposure, or morning melatonin.

Mean PSQI score for all subjects was 5.2 ± 2.7 (range 1 to 15). The subject with a score of 15 was identified as an outlier and excluded from analyses. PSQI scores were not normally distributed, so comparisons were made using the Mann-Whitney test. Mean PSQI was significantly lower for emmetropic subjects (4.2 ± 2.3) than myopic subjects (5.6 ± 2.2, *t stat* = 2.2, P = 0.036), indicating poor sleep quality in the myopic group (PSQI > 5). To understand if poor sleep quality was driven by the high myopes in the group, as previously reported, the myopic group was divided into low myopes (> -0.50 to -4.75, n = 21) and high myopes (> -4.75, n = 10). However, there were no significant differences in the PSQI between the two groups (*t stat* = 1.2, P = 0.22). Regression analysis for PSQI sleep score and morning melatonin showed there was no relationship between these factors (R^2 = 0.007, df = 39, P = 0.61). For the subset of subjects who wore the Actiwatch for one week, mean daily sleep duration between refractive error groups was not significantly different (P = 0.09), with emmetropes sleeping 457.2 ± 38.1 minutes per night, and myopes sleeping 421.7 ± 43.1 minutes per night. Sleep efficiency

was not significantly different between emmetropes (83.3 ± 7.9%) and myopes (85.3 ± 4.3%, $P = 0.50$).

Table 2-2. Age, sex, spherical refraction (SER), axial length, time outdoors (mean minutes per day), white light exposure, sleep duration and efficiency, morning melatonin concentration, and PSQI scores; p value for emmetropes vs myopes; * indicates significance at $p \leq 0.05$

	All subjects	**Emmetropes**	**Myopes**	**P value**
Age (years)	26.9 ± 6.2	27.7 ± 5.5	26.3 ± 6.6	0.50
Sex	26F:24M	9F:10M	17F:14M	1.0
SER (n = 50)	-2.5 ± 2.7 D	+0.38 ± 0.48 D	-4.3 ± 1.6 D	* < 0.005
Axial Length (mm, n = 50)	24.6 ± 1.2	23.6 ± 0.8	25.3 ± 0.9	* < 0.005
Time outdoors (minutes, n = 50)	104.8 ± 46.6	107.6 ± 42.1	103.0 ± 49.9	0.74
White light exposure (log lux, n = 24)	13.99 ± 0.66	14.09 ± 0.66	13.8 ± 0.85	0.42
Sleep duration (minutes, n = 24)	435.5 ± 43.9	457.2 ± 38.1	421.7 ± 43.1	0.09
Sleep efficiency (n = 24)	84.6 ± 5.9%	83.3 ± 7.9%	85.3 ± 4.3%	0.50
Melatonin (pg/ml, n = 43)	6.9 ± 3.5	6.5 ± 2.5	7.1 ± 4.0	0.60
PSQI (n = 50)	5.3 ± 2.7	4.2 ± 2.3	5.9 ± 2.7	* < 0.05

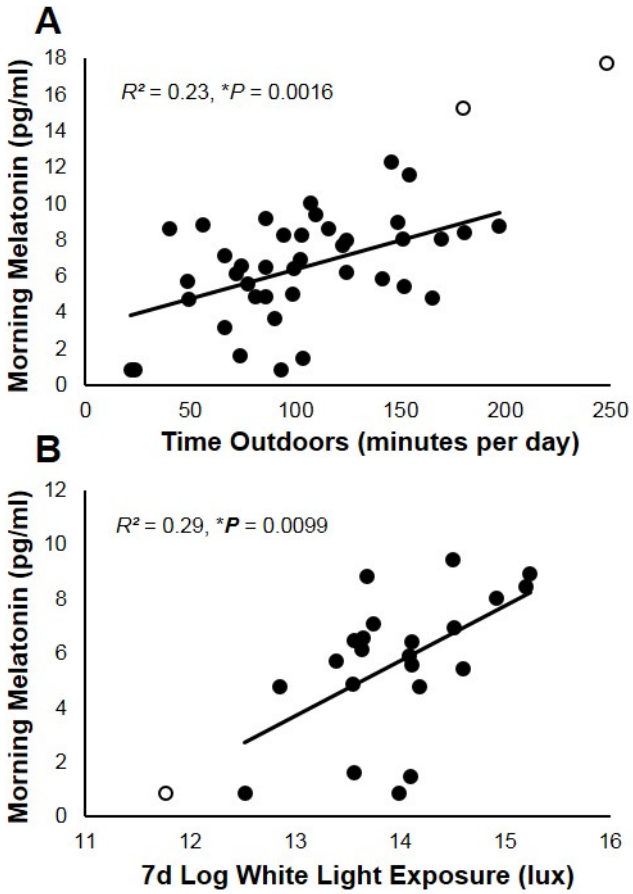

Figure 2-2. A) Correlation between time spent outdoors over the previous week and morning melatonin (n = 43, $P = 0.0016$), and B) objectively measured mean white light exposure over the 7 days and morning melatonin (n = 24, $P = 0.0099$), open symbols represent outliers and are not included in regression analysis, * indicates significance at $P \leq 0.05$

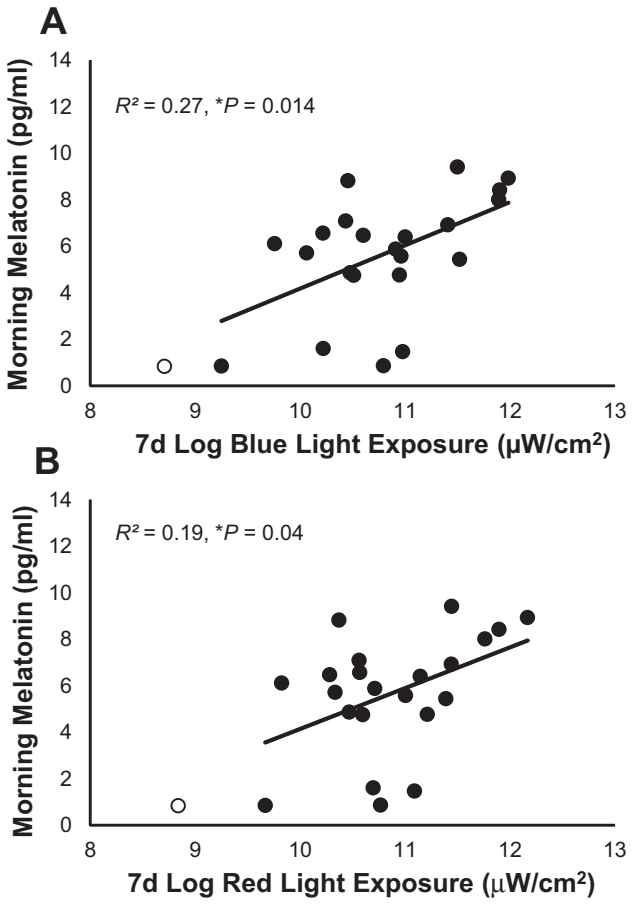

Figure 2-3. Correlation between morning melatonin (pg/ml) and objectively measured (A) mean blue light exposure ($\mu W/cm^2$) and (B) mean red light exposure ($\mu W/cm^2$) over the previous 7 days (n = 24); open symbols represent outliers and are not included in regression analysis; * indicates significance at $P \leq 0.05$

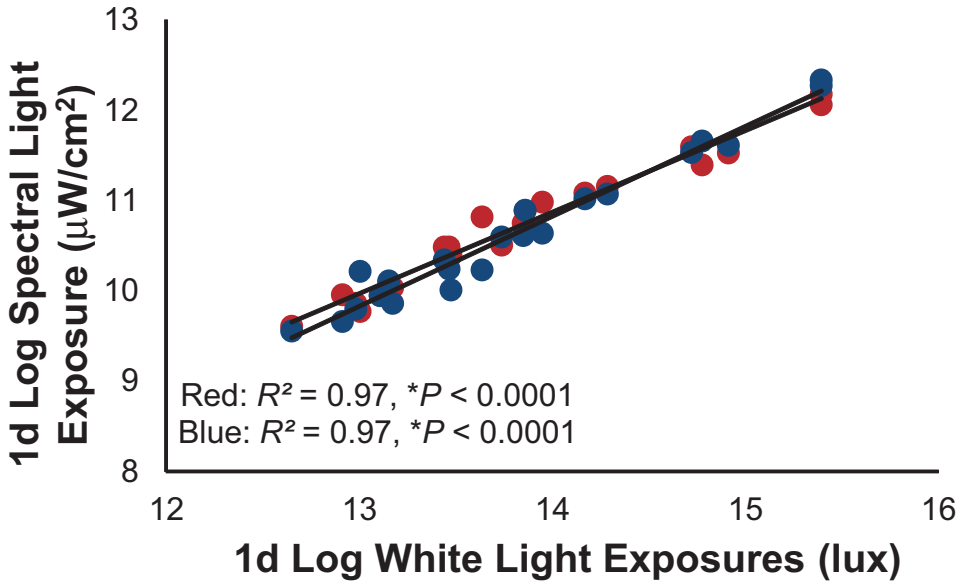

Figure 2-4. Correlations between objectively measured white light exposure (lux) with red and blue light exposure (μW/cm^2, red and blue symbols, respectively), * indicates significance at $P \leq 0.05$

Pupil measurements

Following 5 minutes dark adaptation, the baseline pupil diameter of undilated right eyes was 6.1 ± 0.8 mm (range 3.7 to 7.5 mm), which was not significantly different between the emmetropic (6.10 ± 0.71 mm) and myopic (6.08 ± 0.86 mm) groups (P = 0.95). For long wavelength stimulation, pupils re-dilated rapidly following light offset. For short wavelength stimulation, pupils re-dilated at a slower rate following light offset. These dynamics are evident by the statistically significantly smaller values for the 6 s and 30 s PIPR and larger values for the early and late AUC for 1 s and 5 s to short wavelength stimulation compared to long wavelength stimulation (paired t-test, $P < 0.0001$; Table 2-3), except for the 30 s PIPR to 1 s stimulation, which was not significantly different between long and short wavelength stimulations ($P = 0.10$). All metrics were significantly greater (i.e. lower PIPR values and higher AUC values) for 5 s stimulation versus 1 s stimulation of the same wavelength ($P < 0.01$ for all), except for the 30 s PIPR and late AUC to long wavelength light ($P = 0.09$ and 0.90, respectively).

Trends suggested that the 6 s PIPR to 1 s short wavelength stimulation was associated with white light exposure over the previous day ($R^2 = 0.18$, $df = 23$, $F = 6.7$, $P = 0.05$, Figure 2-5A) and blue light exposure over the previous day ($R^2 = 0.21$, $df = 20$, $F = 5.22$, $P = 0.03$, Figure 2-5C). However, these relationships were not significant following Bonferroni correction to a significance level of $p \leq 0.006$. There was no relationship between red light exposure over the previous day and the 6 s PIPR ($R^2 = 0.07$, $df = 21$, $F = 1.70$, $P = 0.21$, Figure 2-5E). There were no associations between the 6 s PIPR to 5 s short wavelength stimulation and light exposure (Figures 2-5B, 2-5D, 2-5F). Additionally, there were no associations between the 30 s PIPR, early AUC or late

AUC to 1 or 5 s short wavelength stimulation and light exposure (Figures 2-6,2-7,2-8). Pupil metrics to 1 s and 5 s long wavelength stimulation were not associated with light exposure. Pupil metrics were not associated with objectively measured white light exposure over the previous 3, 5, or 7 days (data not shown).

Pupil metrics were not significantly different between emmetropic and myopic subjects, shown for the 1 s stimulus in Figure 2-9A and 5 s stimulus in Figure 2-9B. While the 6 s PIPR to a 5 s short wavelength stimulus was modestly increased for myopes (i.e. lower PIPR value, $t\ stat = -2.0$, $df = 48$, $P = 0.05$), the difference was not significant after Bonferonni correction.

Table 2-3. Pupil metrics for all subjects (n = 50) for 1 s and 5 s long wavelength (red) and short wavelength (blue) stimulations; metrics include peak constriction (% of baseline) during stimulation, 6 s and 30 s post illumination pupil response (PIPR, %), early and late area under the curve (AUC, unitless). * indicates significance at $p \leq 0.05$ for red versus blue stimulations.

Stimulus	Peak constriction	6 s PIPR	30 s PIPR	Early AUC	Late AUC
1 s red	62.1 ± 5.8%	92.6 ± 2.9%	97.2 ± 3.8%	1.22 ± 0.30	0.61 ± 0.62
1 s blue	48.4 ± 10%	68.3 ± 8.8%	95.9 ± 4.9%	3.51 ± 0.75	1.98 ± 1.04
	*p < 0.001	*p < 0.001	p = 0.11	*p < 0.001	*p < 0.001
5 s red	44.8 ± 4.8%	89.5 ± 6.0%	98.5 ± 4.8%	1.70 ± 0.51	0.59 ± 0.90
5 s blue	37.3 ± 3.4%	64.4 ± 8.4%	92.4 ± 5.3%	3.84 ± 0.64	3.02 ± 1.14
	*p < 0.001	*p < 0.001	*p < 0.001	*p < 0.001	*p < 0.001

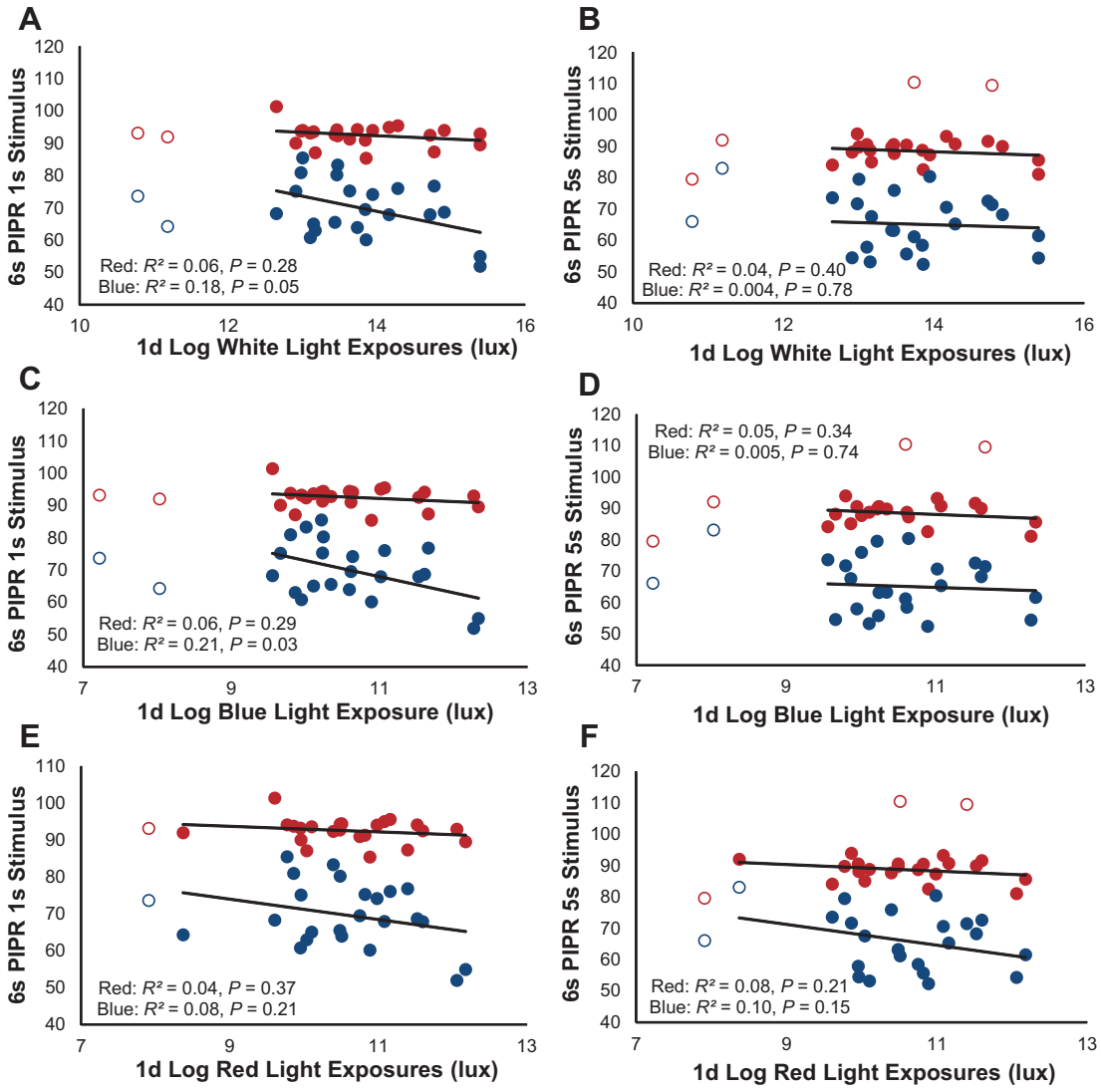

Figure 2-5. 6 s PIPR with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

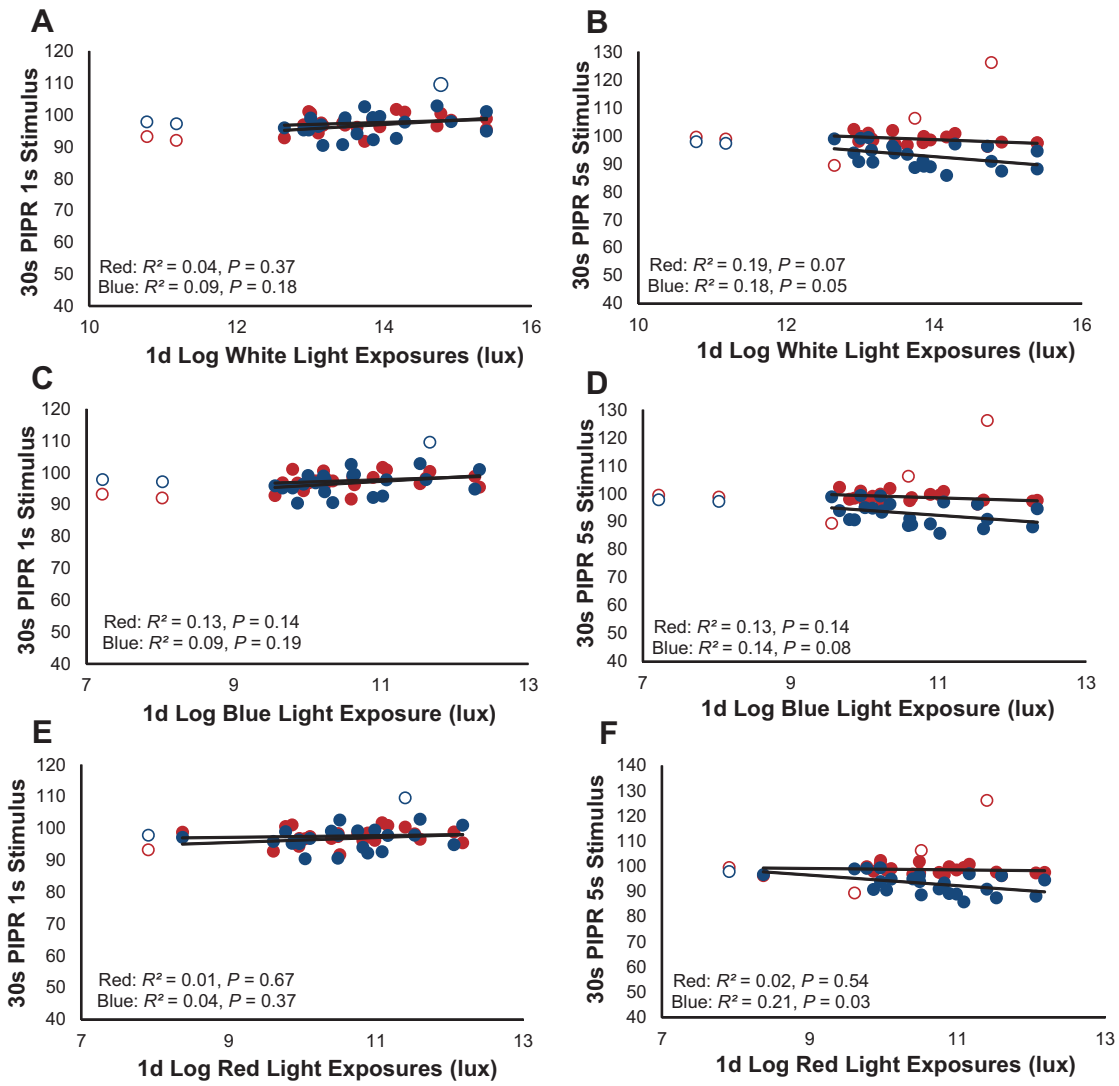

Figure 2-6. 30 s PIPR with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

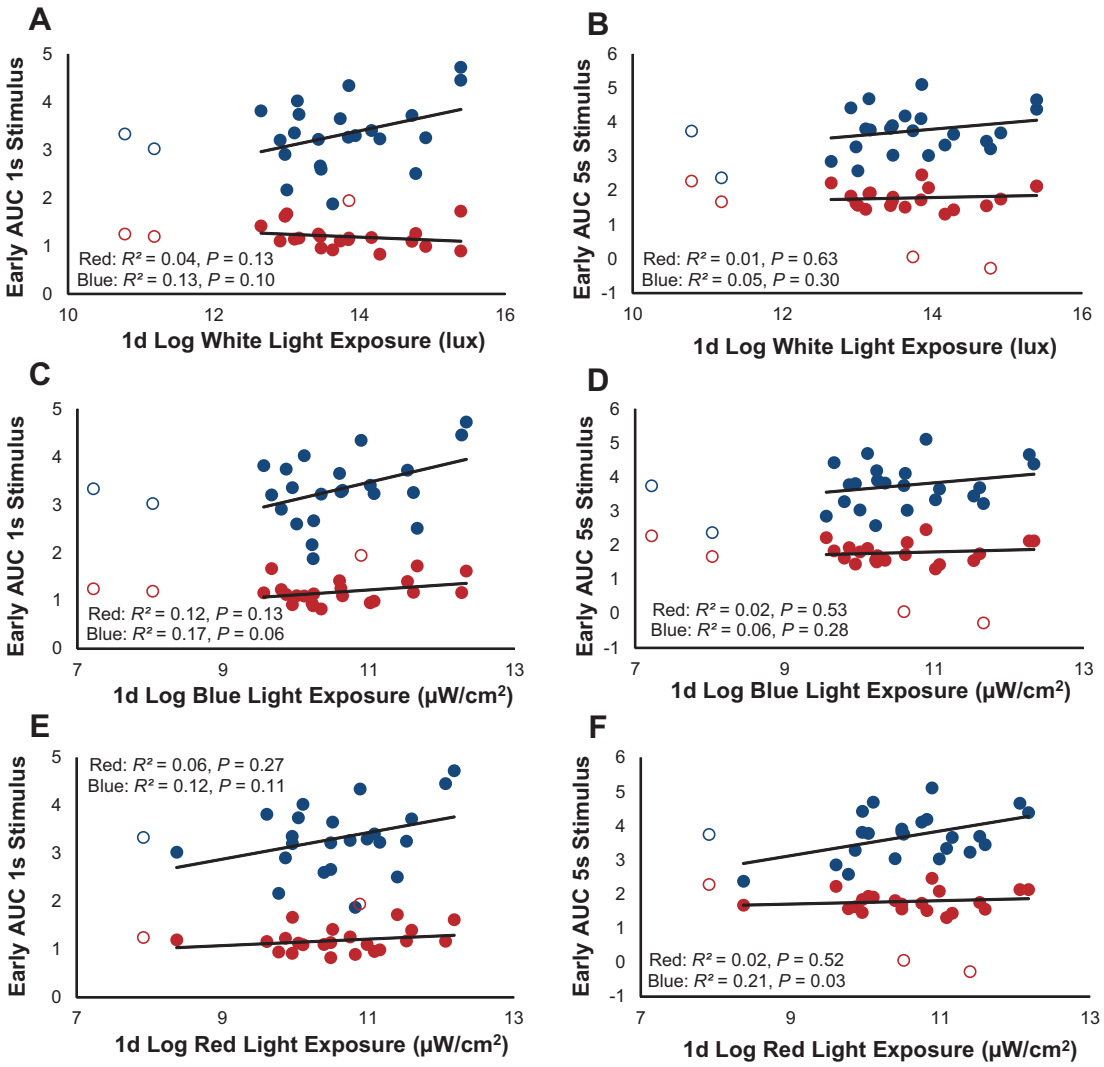

Figure 2-7. Early AUC with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

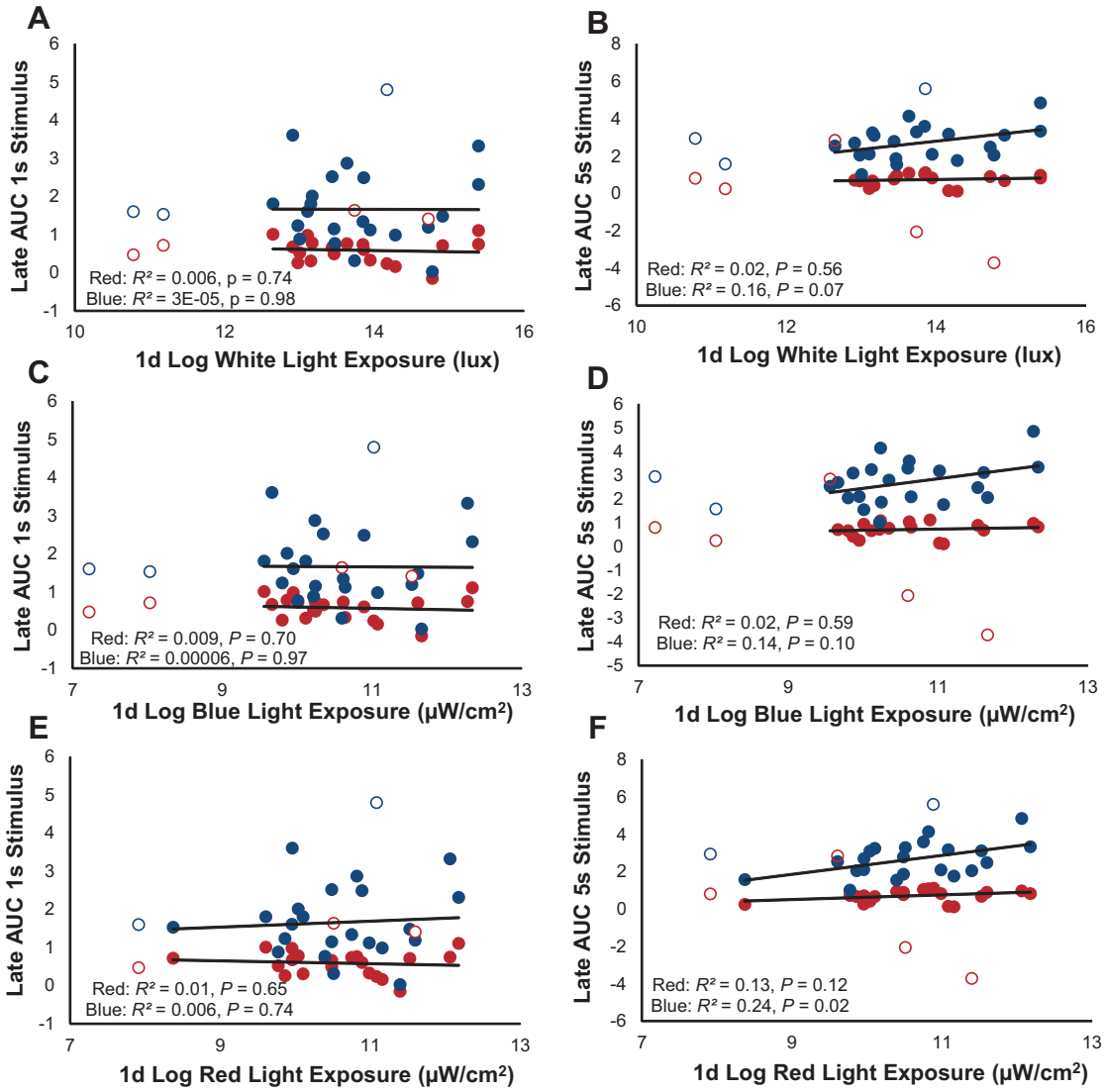

Figure 2-8. Late AUC with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

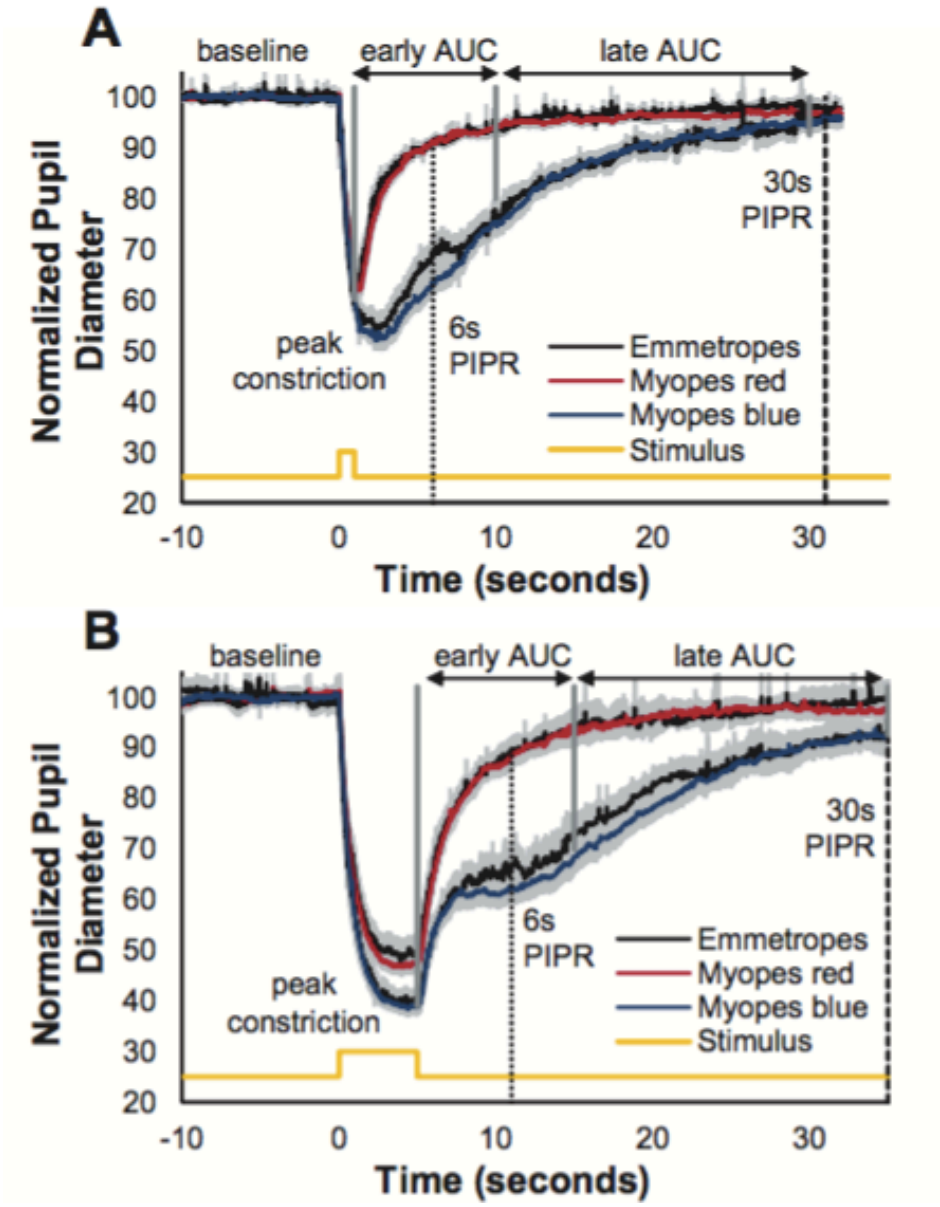

Figure 2-9. Normalized pupil diameter for a A) 1 s pulse and B) 5 s pulse for emmetropes (black traces, n = 19) and myopes (n = 31), overlaid for comparison. Pupil metrics include baseline, peak constriction, 6 s PIPR, 30 s PIPR, early AUC, and late AUC. Shaded regions represent the 95% confidence intervals. Stimulus is shown in yellow.

2.4 Discussion

A primary outcome of this study was that objectively measured habitual light exposure was associated with melatonin concentration. Additionally, our results showed that myopic subjects exhibited poor sleep quality in comparison to emmetropic subjects. Small and inconsistent associations between the ipRGC driven pupil response, light exposure, and refractive error were not significant.

A primary role for ipRGCs is photic regulation of melatonin release from the pineal gland.[113] We demonstrated that systemic morning melatonin concentration was associated with mean time spent outdoors and light exposure over the previous 7 days. However, morning melatonin concentration was not associated with mean light exposure over the previous 1 to 5 days, suggesting that the luminance integration period for melatonin is 5 to 7 days. A potential mechanism for increased morning melatonin with increased habitual light exposure is a phase shift in circadian time. A limitation of the current study as that melatonin was only measured at one time point. Melatonin is known to undergo a diurnal rhythm, in which the systemic levels increase at nighttime, and decrease in the morning.[206] Measuring melatonin at multiple time points throughout a twenty-four hour period would address whether a phase shift occurs with increased light exposure. Our results are consistent with a recent study reporting higher morning melatonin levels with increased daytime light exposure in the elderly.[207] While the age of the current subjects is younger, sampling methodology was similar between studies. Another recent study showed that melatonin levels were higher in summer compared to winter;[208] summer has a longer photoperiod and, hence, likely greater light exposure. Previous studies evaluated brief exposure to bright light on systemic melatonin. Lewy et

al., demonstrated an immediate suppression of melatonin during bright light at nighttime that returned to normal values after darkness was resumed.[121] Owen and Arendt showed that light suppression of melatonin was more effective in the latter part of the night.[209] These studies suggest that time of day of light exposure plays an important role in how melatonin is affected. Because of the variability in when our subjects received high light doses over the observational week, we were unable to draw conclusions based on time of day.

Consistent with the literature, pupil re-dilation was slower following short wavelength stimuli compared to long wavelength stimuli, as indicated by the smaller 6 s PIPR and larger early and late AUC values for short wavelength stimuli. Our results showed trends that increased light exposure over the previous day was associated with the amplitude of the PIPR to a 1 s short wavelength light stimulus, as measured at one consistent time point in the morning, suggesting increased morning ipRGC activity, or a shift in circadian phase, with increased habitual light exposure. While this finding did not reach statistical significance following correction for multiple comparisons, the trend is in accordance with a previous study that showed slowed pupillary redilation (i.e. increased ipRGC activity) to 0.1 Hz flashing stimuli with greater habitual light exposure.[200] Further studies with a larger sample size should be carried out to address the significance of these findings. It is of interest to note that, here, the observed trends were evident for both white and blue habitual light exposure, but not for red light exposure, providing further evidence that a driving input for the ipRGCs is short wavelength light. Other studies evaluated circadian changes in the ipRGC driven pupil response over 24 hours in controlled lighting environments, and found that pupil responses demonstrated circadian

modulation that was under the influence of an endogenous circadian clock.[78, 89] Our results suggest that, while relative pupil function undergoes circadian modulation independent of external light cues, the absolute values may be influenced by previous light exposure which could explain variability between subjects.

Pupil size is influenced by several factors in addition to ipRGC activity, including age, lethargy, and autonomic input.[210] While intrinsic contributions to iris musculature cannot be eliminated, efforts were taken in our protocol to minimize input, including using a controlled dark environment, presenting a fixation target that with minimal stimulus for accommodation, and performing measurements at a consistent time of day (i.e. morning). Pupil diameters were normalized to each subject's baseline pupil diameter following 5 minutes of dark adaptation to account for variations in baseline pupil size.[86] With normalization to baseline pupil size, Zele et al. showed that ipRGC-driven PIPR was independent of age.[90] On the other hand, Herbst et al., demonstrated that some metrics of the ipRGC-driven pupil response were associated with age, and attributed this to increased scatter by the lens with age.[86] Our results also found no relationship between the PIPR and age; however, our population included a limited age range up to 40 years.

Light is a potent cue for circadian rhythm entrainment, and studies show that phototransduction through the ipRGCs plays an important role in setting the circadian clock.[10] Studies in chicks,[211] tree shrews[212] and rhesus monkeys[213] have shown that a diurnal light:dark photoperiod is essential for normal refractive development. Li et al., showed that at least 4 hours of darkness is required in chicks for normal emmetropization.[214] Additionally, high ambient light levels decreases experimental myopia in chicks.[215, 216] Results from these animal studies indicating that illumination

level and periodic light:dark cycles are critical for normal refractive development suggest that intrinsic circadian clock and dopamine/melatonin pathway play a role in eye growth through the ipRGCs.[217]

Previous studies have shown that, in an adult population, objectively measured outdoor time is not significantly related to refractive error in adult populations.[202] However, evidence suggests that time outdoors is protective for myopia in children.[187, 218] To date, the relationship between the ipRGC-driven pupil response and light exposure has not been measured in children. The protective effects of outdoor light against myopia may be due to a variety of factors. High intensity light exposure experienced outdoors increases retinal dopamine and also directly stimulates the ipRGCs, which have synaptic connections to dopaminergic amacrine cells.[29] Dopamine has been shown to regulate mRNA expression of melanopsin in ipRGCs.[219] Decreased dopamine has been shown to induce myopia in mice,[181] while dopamine agonists, such as apomorphine, inhibit experimental and spontaneous myopia in chicks and guinea pigs.[220, 221] Another possible protective mechanism of light on myopia include increases in vitamin D.[222] Optical factors may play a role as well, such as a decrease in aberrations due to a smaller pupil size with high intensity light. Additionally, outdoor scenes provide an infinite viewing distance and little requirement for accommodation.

Here, myopic subjects reported poorer sleep quality than emmetropic subjects using the PSQI. These results are in accordance with previous reports that myopic children and young adults have poor sleep quality and shorter duration compared to emmetropes.[189, 190] Several potential mechanisms may link behavioral and circadian aspects of sleep with refractive error. Myopia has been shown to be associated with

education,[184] which may be related to longer hours studying and later bed times. Circadian rhythms influenced by light exposure subserving sleep/wake patterns may also play a role in myopia development. Previous studies have shown that law students with less than 5.6 hours of darkness may progress in myopia at a faster rate than those with more hours of darkness.[223] In animal studies, disruptions in normal light dark patterns also induce refractive errors.[224]

A recent study reported higher morning melatonin in myopic subjects compared to emmetropic subjects.[191] Similar trends were observed here; however, differences did not reach significance. The previous study measured serum melatonin, while in this study, melatonin was assessed via a salivary assay, which may have more variability in melatonin quantification. A higher morning melatonin concentration would contribute to subjectively decreased daytime wakefulness and alertness, and is consistent with the finding of poorer scores in myopic subjects from the Pittsburg Sleep Quality Index reported here.

In conclusion, we demonstrated that systemic melatonin is influenced by light exposure over the previous 7 days. Metrics of the ipRGC-driven pupil response showed small and inconsistent associations with light exposure and refractive error. Myopic subjects exhibited poor sleep quality compared to emmetropic subjects. Results suggest that complex relationships exist between light exposure, ipRGCs, and with potential downstream effects on refractive error and sleep.

2.5 Acknowledgements: This work was supported by NIH NEI P30 EY007551 and NIH T35 EY07088. Special thanks to Alexander Schill for measurement of the stimuli properties.

Presented in part at the 2016 annual American Academy of Optometry meeting (Anaheim, CA), received award to KA for best student presentation

Chapter Three

Attenuation of Short Wavelengths Alters Sleep and the ipRGC Pupil Response

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Abstract

Purpose: Exposure to increasing amounts of artificial light during the night may contribute to the high prevalence of reported sleep dysfunction. Release of the sleep hormone melatonin is mediated by the intrinsically photosensitive retinal ganglion cells (ipRGCs). This study sought to investigate whether melatonin level and sleep quality can be modulated by decreasing nighttime input to the ipRGCs.

Methods: Subjects (ages 17-42, n=21) wore short wavelength-blocking glasses prior to bedtime for two weeks. The ipRGC-mediated post illumination pupil response (PIPR) was measured before and after the experimental period. Stimulation was presented with a ganzfeld stimulator, including 1 second (s) and 5 s long and short wavelength light, and the pupil was imaged with an infrared camera. Pupil diameter was measured before, during and for 60 s following stimulation, and the 6 s and 30 s PIPR and area under the curve (AUC) following light offset were determined. Subjects wore an Actigraph device for objective measurements of activity, light exposure, and sleep. Saliva samples were collected to assess melatonin content. The Pittsburgh Sleep Quality Index (PSQI) was administered to assess subjective sleep quality.

Results: Subjects wore the blue-blocking glasses 3:57 ± 1:03 hours each night. After the experimental period, the pupil showed a slower redilation phase, resulting in a significantly increased 30 s PIPR to 1 s short wavelength light, and decreased AUC for 1 s and 5 s short wavelength light, when measured at the same time of day as baseline. Night time melatonin increased from 16.1 ± 7.5 pg/mL to 25.5 ± 10.7 pg/mL ($p < 0.01$).

Objectively measured sleep duration increased 24 minutes, from 408.7 ± 44.9 to 431.5 ± 42.9 minutes ($p < 0.001$). Mean PSQI score improved from 5.6 ± 2.9 to 3.0 ± 2.2.

Conclusions: The use of short wavelength-blocking glasses at night increased subjectively measured sleep quality and objectively measured melatonin levels and sleep duration, presumably as a result of decreased nighttime stimulation of ipRGCs. Alterations in the ipRGC-driven pupil response suggest a shift in circadian phase. Results suggest that minimizing short wavelength light following sunset may help in regulating sleep patterns.

3.1 Introduction

Intrinsically photosensitive retinal ganglion cells (ipRGCs) in the inner retina are photosensitive and directly stimulated through activation of the photopigment melanopsin. Melanopsin stimulation through this intrinsic pathway is most sensitive to short wavelength light, with a peak sensitivity of ~482 nm.[10, 15] The ipRGCs are also stimulated synaptically through the rod/cone pathway (the extrinsic pathway).[26] ipRGCs are primarily involved in non-image forming processes such as circadian rhythm entrainment and pupil size regulation,[41, 58] and can be considered irradiance detectors. They are linked to various aspects of circadian rhythm via the retinohypothalamic tract.[15, 16, 225] In humans, two subtypes of ipRGCs with distinct morphology and axonal projections have thus far been identified,[15, 50, 51] with a recent study reporting four subtypes (M1-M4).[52] One subset of ipRGC axons projects to the suprachiasmatic nucleus (SCN), in a pathway that ultimately leads to the pineal gland to control melatonin release.[22] Melatonin is a hormone released in dim light and involved in the physiological control of sleep.[226]

Intrinsic, melanopsin-driven ipRGC activity can be measured through the post illumination pupil response (PIPR).[58, 227] With high intensity short wavelength light, a rod/cone and ipRCG driven pupil constriction ensues, which continues for up to 3 minutes following light offset.[15, 204] Known circuits suggest that pupil dynamics following light offset are mediated by sustained firing of ipRGCs.[15, 58, 228]

Light is a potent cue for entraining the circadian system, and has been shown to affect a wide variety of physiologic functions, including potential roles in cardiovascular, metabolic, endocrine, and neurologic systems.[229-235] Light exposure during the night can

lead to chronodisruption, or impaired physiological, behavioral, and biochemical rhythms.[118] While other cues for synchronization of the circadian clock include feeding and physical activity, light alone is sufficient to synchronize circadian processes.[236]

Evening exposure to short wavelength light prior to bedtime may disrupt sleep wake cycles through ipRGC-induced melatonin suppression,[112, 237] contributing to the high frequency of reported incidence of sleep dysfunction, shown to affect up to 40% of the population.[238, 239] Software has recently been introduced which aims to reduce blue light exposure from electronic devices at night;[164] however, computers and handheld devices represent only a portion of the artificial light in the environment. Exposure to high intensity short wavelength light increases sleep latency, as measured by EEG.[128, 240] Additionally, nighttime exposure to backlit computer screens attenuates salivary melatonin levels.[241] Subjective improvements in both sleep quality and mood,[165] as well as a decrease in LED-induced nighttime melatonin suppression,[166] have been demonstrated in subjects wearing blue-blocking glasses at nighttime for two weeks. Short-wavelength blocking glasses have also been shown to prevent melatonin suppression from bright light during simulated shift work at night.[242]

This study sought to investigate whether previously observed improvements in sleep following evening wear of blue blocking glasses is mediated through the ipRGCs. The PIPR was utilized as an indirect measure of ipRGC activity to understand its contribution to systemic melatonin and sleep patterns. Based on evidence from previous studies, we hypothesized that attenuating nighttime blue light stimulation would result in increased melatonin and improved sleep, potentially complemented with an increase in the PIPR.

3.2 Methods

Subjects

Twenty-two subjects, ages 17-42, were recruited to participate in this study. All lab visits occurred between 9:00 am to 11:30 am to minimize circadian influences on the PIPR.[78] Visual acuity was measured with habitual correction and an anterior eye exam using slit lamp biomiscroscopy was performed to confirm suitability for dilation. All subjects had a visual acuity of 20/25 or better. Exclusion criteria included ocular pathology, including cataracts, prescription or over-the-counter medications known to affect sleep or the pupil, sleep aids such as melatonin, and shift work or travel across time zones during the previous month. Methods were approved by the University of Houston institutional review board and carried out in accordance with relevant guidelines. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained after explaining the nature of the study to subjects.

The experimental protocol is shown in Figure 3-1A. As described in detail below, melatonin level and the PIPR were measured before and after 2 weeks of wearing short wavelength-blocking glasses before bedtime. In addition, light exposure, activity, and sleep were objectively and continuously monitored before and during the experimental period.

Objective light exposure, activity and sleep monitoring

Subjects wore an Actigraph device (Actiwatch Spectrum, http://www.usa.philips.com) for one week prior to the first lab visit and during the 2 week experimental period for objective and continuous measurements of activity, light

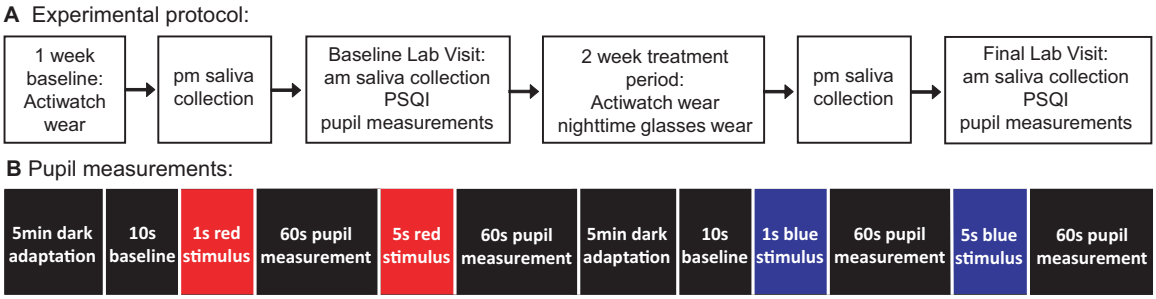

Figure 3-1. (A) Flow chart of the three week experimental protocol (B) For pupillometry, subjects dark adapted for 5 min. Baseline pupil diameter was recorded for 10 s, then a 1 s long wavelength (red) stimulus was presented followed by 60 s recording, and a 5 s long wavelength stimulus was presented followed by 60 s recording. Subjects dark-adapted again, and the protocol was repeated with a short wavelength (blue) stimulus.

exposure, sleep quality, and sleep duration. The Actiwatch Spectrum is a wrist worn Actigraph device that measures ambient light exposure and activity continuously at 32 Hz, and was set to average over 1 minute epochs. The light sensor consists of a photodiode that measures the illuminance of broad band light in units of lux (range 0.1 - 200,000 lux). Additionally, three color sensitive diodes measure the irradiance of red (600-700 nm), green (500-600 nm) and blue (400-500 nm) spectral components. The battery life and memory allow for continuous wear over the entire three week study period. Subjects were asked to not remove the device for the entire experimental period, and compliance was monitored by an off-wrist sensor in the device.

Melatonin analysis

A saliva sample was collected the night before each lab visit, just before the subject's habitual bedtime, and the morning of the lab visit, for subsequent melatonin analysis;[243] samples were collected within 15 minutes of each other for baseline and final time points. Prior to collection, subjects were instructed not to eat, drink any liquids other than water, or brush their teeth within an hour of collection. In addition, subjects were asked to abstain from alcohol and nicotine 12 hours prior to collection. Subjects collected approximately 1 ml of saliva, and the vial was immediately placed in the freezer. For nighttime collections that were done at the subject's home, the sample was brought into lab in a provided thermos and insulated bag. Samples were stored at -20° C for subsequent analysis using a melatonin ELISA kit (Salimetrics, https://www.salimetrics.com). All samples were run in duplicate.

Subjective sleep quality

The Pittsburg Sleep Quality Index questionnaire (PSQI) was administered as a subjective measure of sleep quality. PSQI scores distinguish "good" vs "poor" sleepers based on seven different sleep components, with lower scores indicating better sleep quality.[244] A score of 5 or greater indicates poor sleep quality. Subjects answered the questionnaire at baseline, with respect to their habitual sleep over the last month (as the survey indicates), and after 2 weeks, with the latter survey being answered with respect to the 2 week experimental period.

Pupillometry

The ipRGC-driven PIPR was measured at the baseline lab visit and repeated at the 2 week follow up visit. The left eye was dilated with 2.5% phenylephrine and 1% tropicamide, and a frame mounted binocular eye tracker with infrared illumination (ViewPoint EyeTracker, http://www.arringtonresearch.com) was used to record the pupil size of the right eye at 60 Hz. The IR LED light source has a lambda max of 943 nm with a half-max width of 46 nm (Ocean Optics Spectrometer, https://oceanoptics.com, spectrum provided in Figure 3-2). Pupil diameter was calibrated for each subject by capturing an image of a 5 mm printed black circle approximately placed at the cornea plane. Following calibration, subjects dark adapted for 5 minutes (< 0.1 lux), then placed their head in a chinrest with an LED-driven Ganzfeld system (Color Burst, Espion, Diagnosys LLC, http://diagnosysllc.com) centered in front of the left eye at 10 mm, providing full field stimulation. The subject viewed a red fixation point with the right eye during measurements, which was on the wall 10 feet away.

Stimuli presented to the left eye consisted of 1 second (s) and 5 s pulses of either long wavelength (red) or short wavelength (blue) light (Figure 3-1B). Long wavelength stimuli were 651 nm with a half-max width of 25 nm (Spectroradiometer CS1W, http://sensing.konicaminolta.us, spectral output provided in Figure 3-3 and 3-4), set to 33.3 cd/m^2, and with a measured corneal irradiance of 5.58 x 10^{13} photons/cm^2/s (Power Meter, https://www.newport.com). Short wavelength stimuli were 456 nm (half-max width of 20 nm), set to 16.67 cd/m^2, with a measured corneal irradiance of 5.85 x 10^{13} photons/cm^2/s. At this irradiance, with full field ganzfeld stimulation at a distance of 10 mm, the intensity was above the melanopsin threshold,[15, 245] as indicated by reduced pupil

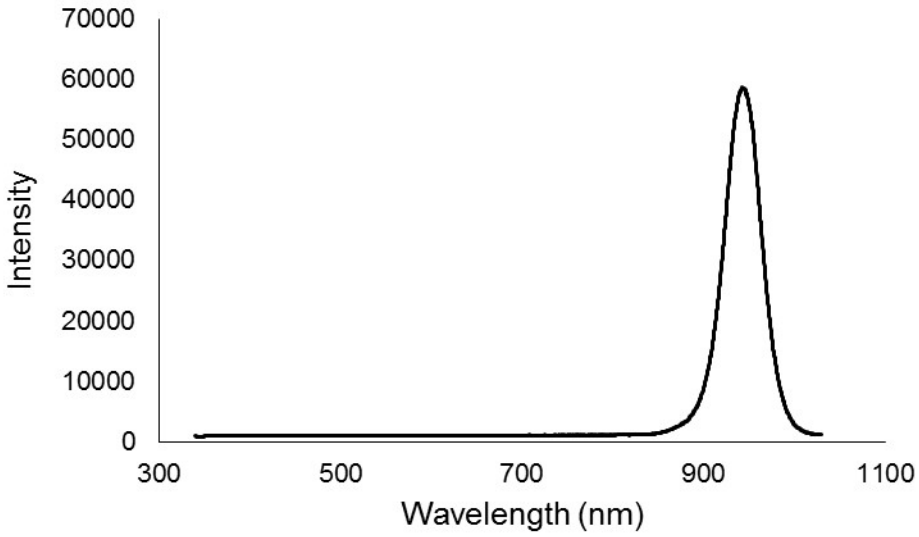

Figure 3-2. Output from the infrared light source. The output from the infrared light source is shown in figure 2-2. The IR LED light source had a lambda max of 943 nm with a half-max width of 46 nm (Ocean Optics Spectrometer, FL, USA).

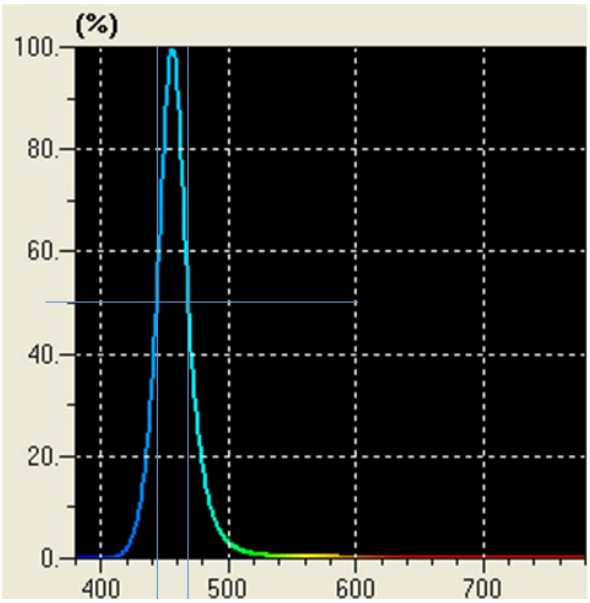

Figure 3-3: Normalized spectral output for the short wavelength stimulus

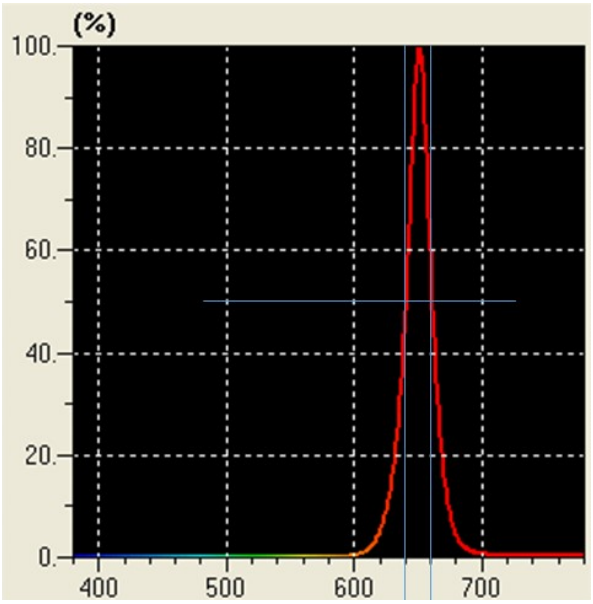

Figure 3-4: Normalized spectral output for the long wavelength stimulus

Figure 3-3 and 3-4. The normalized spectral output is shown for the short wavelength stimulus (3) and long wavelength stimulus (4), as measured with a Minolta Spectroradiometer CS1W.

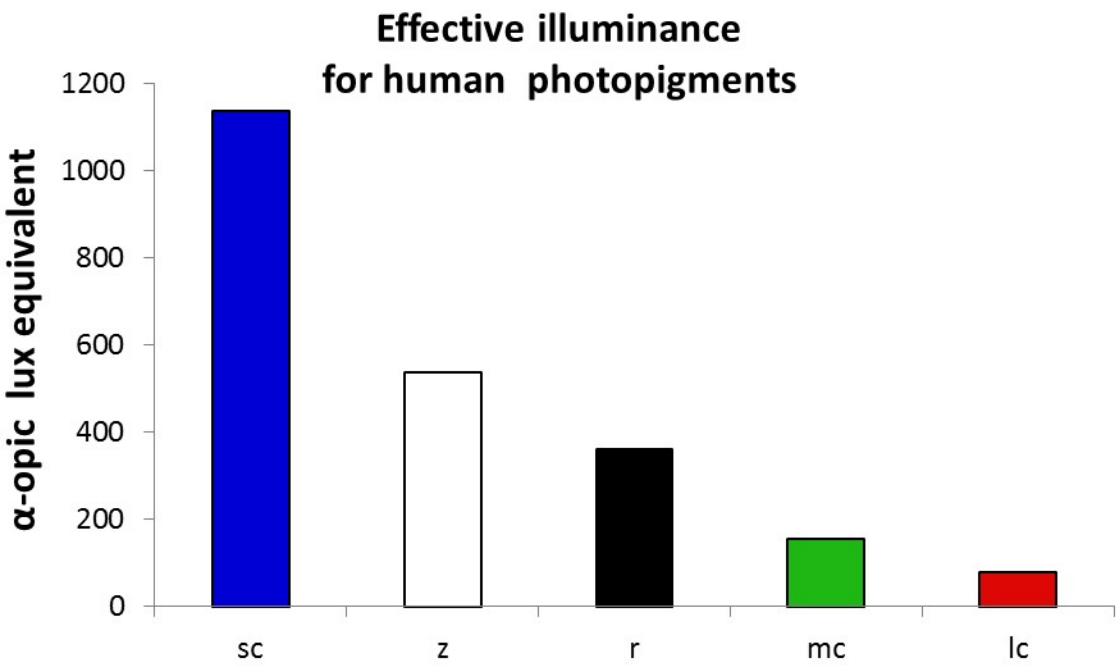

Figure 3-5. Short wavelength stimulus alpha optic lux equivalent. The toolbox provided by Lucas, et al, 2014, was utilized to determine the alpha-opic luminance of the long and short wavelength stimuli used here. The short wavelength stimulus alpha-opic lux equivalent is shown in figure 2-5, and the long wavelength stimulus is shown in figure 2-6 (see table 3-1), where sc = s cone z = melanopsin r = rod mc = m cone lc = l cone

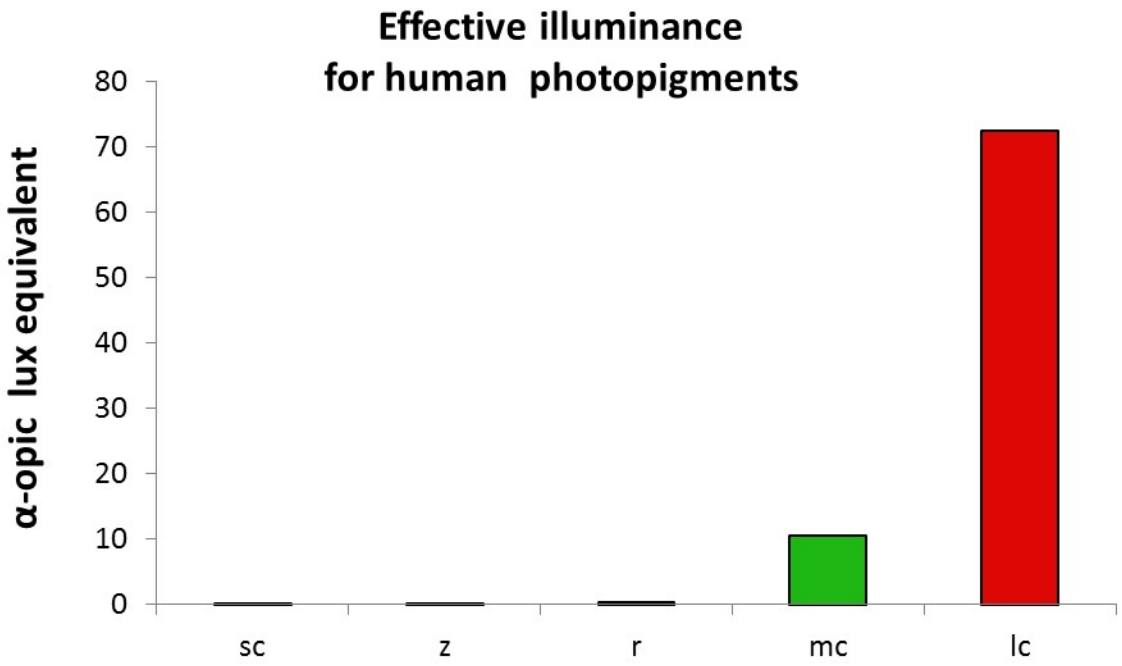

Figure 3-6. Long wavelength stimulus alpha optic lux equivalent

Table 3-1. Alpha-optic lux for the short and long wavelength stimuli used in this study

Sensitivity	lmax	α-opic lux	
		short λ stimulus	long λ stimulus
S cone	419.0	1,136.66	0.00
Melanopsin	480.0	537.75	0.06
Rod	496.3	362.40	0.42
M cone	530.8	154.82	10.63
L cone	558.4	77.72	72.57

diameter measured at 6 s and 30 s following short wavelength stimulus offset compared to long wavelength stimuli. Excitation for each photoreceptor class is estimated using the toolbox provided by Lucas et al, which shows that the differential melanopsin activation between short and large wavelengths is large.[246]

Stimulus parameters included 2 durations, 1 s and 5 s. Previous studies have used a range of stimulus durations from 4 ms to 30 s,[79, 204] and while it has been concluded that a 1 s stimulus is sufficient to stimulate the melanopsin pathway, the 5 s stimulation was included here to understand if PIPR metrics would be different for longer stimulations, as the ipRGCs have been shown to continue firing for the duration of a stimulus,[58] while rod and cone activity decays over time.[247] The order of stimulus presentation was consistent from baseline to follow up so that direct comparisons could be made. Following dark adaptation, the baseline pupil size was recorded in the dark for 10 s. A long wavelength 1 s pulse was presented to the left eye, and the pupil diameter of the right eye was measured for 60 s. Then a long wavelength 5 s pulse was presented to the left eye and the response of the right eye was again measured for 60 s. The subject dark adapted for 5 minutes, and the protocol was repeated with short wavelength light. Previous evidence suggests that prior long-wavelength light exposure enhances short-wavelength induced pupil constriction.[205] Therefore, both long wavelength stimuli were presented, followed by a dark adaptation period, and then blue stimuli were presented, similar to previously published experiments.[89] One measure was recorded for each stimulus duration, as the melanopsin response can persist for up to 3 minutes,[204] and attempts were made to avoid potentiation of the response.[248] This portion of the protocol took 16 minutes, including

the two 5 minute adaptation periods. Because of the short length, subject fatigue was not a concern.

Experimental period

Short wavelength-blocking glasses (Ultraspec 2000, https://www.uvex.com/en) were dispensed for the subjects to wear in the evenings for 2 weeks. The transmission spectrum of the glasses was confirmed, showing that the lenses absorb approximately 99% of light shorter than 540 nm, and transmit approximately 90% longer than 540 nm (Humphrey Lens Analyzer, https://www.zeiss.com, transmission spectrum provided in Figure 3-7). All experiments took place in the summer and fall, in which sunset occurred prior to 8:00 pm (Earth System Research Laboratory, https://www.esrl.noaa.gov/gmd). Subjects were asked to wear the glasses from 8:00 pm until the lights were turned off in their home for bedtime, unless they habitually went to bed prior to 11:00 pm, in which case they were asked to put them on 3 hours before bedtime. Therefore, all subjects wore the glasses for at least 3 hours, although some wore them longer based on their habitual bedtime. In addition, subjects were asked to wear the glasses if they awoke during the night (any time before sunrise), before using any electronic devices or turning on a light. Subjects were advised to set an alarm reminding them to wear the glasses each night, and compliance was monitored by subject report.

Data Analysis

Pupil data were analyzed off line using custom written software (MATLAB, https://www.mathworks.com). Pupil diameter measurements were sampled at 60 Hz. The

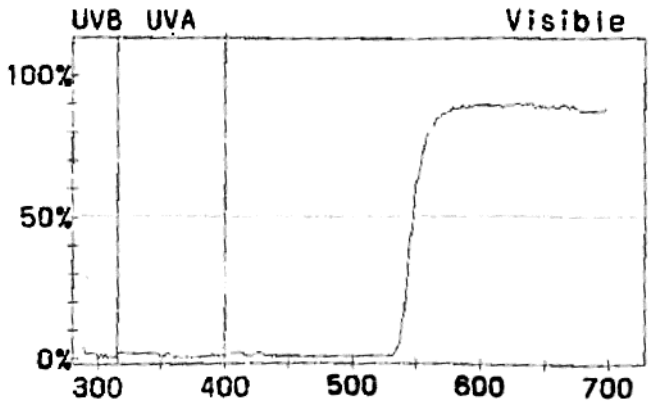

Figure 3-7. The transmission spectrum of the blue-blocking glasses is shown in figure 3-5, as measured with a Humphrey Lens Analyzer (https://www.zeiss.com).

raw data included extreme, fast excursions of pupil diameter due to blinks, when the video frame of the ViewPoint system was not able to capture the pupil. The samples that the ViewPoint system identified as poor quality were removed. This strategy did not remove all extreme excursions, so additional filtering was applied. Samples were removed if they were less than 1 mm, if the rate of change was outside 1 standard deviation from the mean, or if the recorded pupil aspect ratio was outside 1 standard deviation from the mean. This strategy left very few excursions in the data.

Three metrics were used to evaluate the PIPR, which have been used in previous studies, and are useful in describing various parameters of the PIPR. Metrics included the 6 s PIPR, 30 s PIPR, and area under the curve (AUC). The PIPR 6 s after stimulus offset is commonly used in the literature to describe pupil redilation and takes into account inter-subject variability and age related effects in baseline pupil size, as values are normalized to each subject's baseline pupil diameter.[79, 249] The 30 s PIPR provides further information on duration of ipRGC activity, as the PIPR can be sustained up to 83-180 s depending on stimulus duration.[204] We found that the pupil diameter oscillated during redilation following short wavelength stimulation, and the 6 s PIPR value could fall on an oscillation, increasing the noise inherent in PIPR measurements. Therefore, the AUC was also calculated,[250] which captures the influence of these "melanopsin oscillatory responses (mORs)," or hippus, in pupil diameter seen during the redilation phase. Pupil metrics were calculated as follows (Table 3-2, Figure 3-8):

6 s and 30 s PIPR: Pupil diameter was normalized to baseline, setting the average baseline pupil diameter during the 10s prior to stimulation to 1. Relative pupil diameter

was averaged over 6-7 s after stimulus offset and 30-31 s after stimulus offset for the retained samples following filtering.

Table 3-2. Pupil metrics. Key. s: seconds; PIPR: post illumination pupil response; AUC: area under the curve.

Metric	**Calculation**
Baseline pupil diameter	Average pupil diameter 10s prior to light stimulation
Maximum constriction	Minimum pupil diameter during light stimulation
6 s PIPR	Pupil diameter averaged over 6-7 s after stimulus offset, relative to the baseline pupil diameter
30 s PIPR	Pupil diameter averaged over 30-31 s after stimulus offset, relative to the baseline pupil diameter
AUC	Trapezoidal sum of the interpolated normalized trace for 20s after light offset

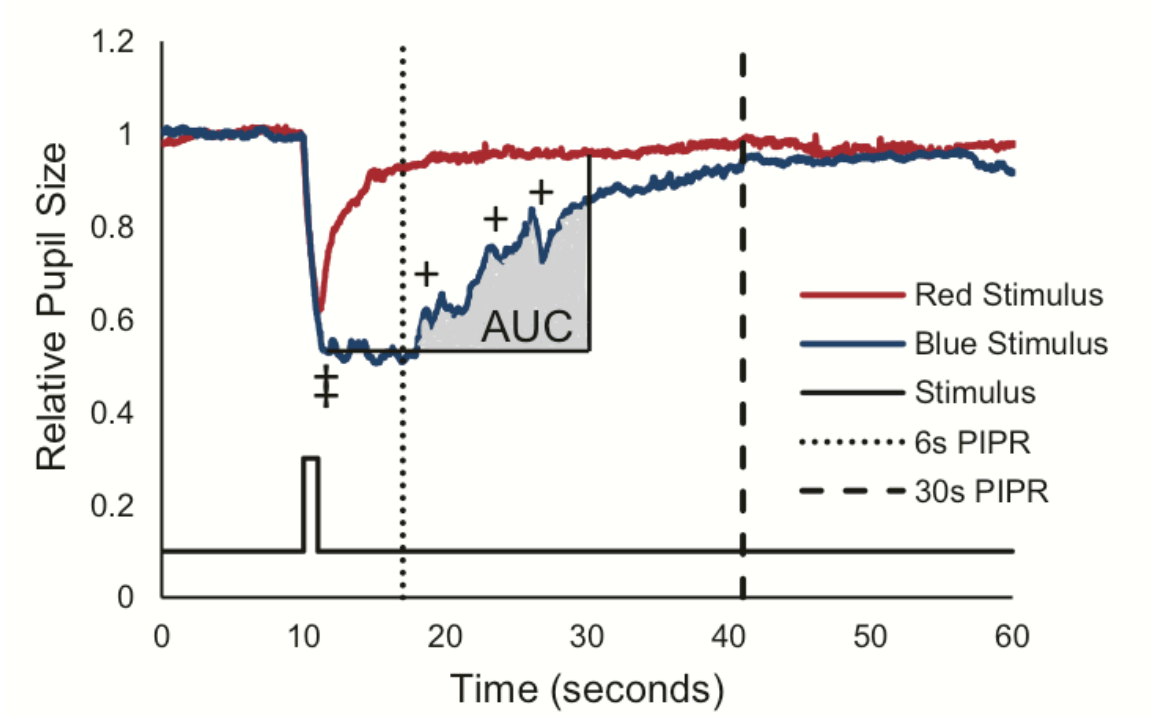

Figure 3-8. Pupil diameter of the right eye during a 1 s long wavelength stimulus (red trace) presented to the left eye, overlaid with the pupil diameter during a 1 s short wavelength stimulus (blue trace) for one representative subject. ‡ indicates the maximum pupil constriction, dotted and dashed lines show where the 6 s and 30 s PIPR (post illumination pupil response) are measured, + indicates the melanopsin oscillatory responses. The area under the curve (AUC) for the blue stimulus is shaded grey, and is calculated with respect to the normalized pupil size.

Area under the curve (AUC): AUC was computed as the trapezoidal approximation of the integral of the interpolated, normalized trace for 20 seconds following light offset. The units are normalized pupil diameter times seconds.
For each of these metrics, a lower number indicates slower redilation following light offset and hence, increased melanopsin-driven ipRGC activity.

Data from the Actiwatch were downloaded and analyzed with Actiware software (Actiware 6.0.4, http://www.usa.philips.com), which calculated mean daytime activity (counts per minute, CPM) and sleep latency, duration, and efficiency for each day. Values were averaged separately for the baseline week and for the 2 week experimental period. Ambient illumination values greater than 1000 lux were classified as outdoors, as in previously published studies,[202, 218, 251] and light exposure analyses were performed using log average daily illumination.[188]

Statistical analyses were performed with R (R Core Team 2105, https://www.r-project.org). For PIPR metrics, analyses were performed on normalized values. Shapiro-Wilk test showed that melatonin levels, PIPR metrics and log light exposure values were normally distributed and therefore, were analyzed with simple linear regressions. Outlier analyses for melatonin levels and light exposure were performed with a modified Thompson tau test. Melatonin levels, pupil diameters, and Actigraph parameters from baseline and the experimental period were analyzed with paired two-tailed t-tests. P values less than 0.05 were considered statistically significant. Values are expressed as mean ± standard deviation.

3.3 Results

Of the 22 subjects, one subject was excluded due to non-compliance wearing the Actiwatch and the glasses. Actiwatch data indicated that the remaining subjects did not remove the watch during the experimental period, and hence, 21 were included in analyses. Subjects consisted of 10 females and 11 males, with an average age of 26.7 ± 7.8 years (range 17.4 – 39.7 years). Mean wear time of the glasses was 3:57 ± 1:03 hours per night.

Actigraphy, Melatonin and Sleep Analysis

During the baseline week, subjects spent an average of 93.6 ± 44.7 minutes outdoors per day, which was not significantly different from the 2 week experimental period, 93.1 ± 43.0 minutes outdoors ($p = 0.93$). Subjects received a similar amount of daily light exposure during the baseline week and experimental period (1.4×10^6 lux per day, $p = 0.83$). For salivary melatonin analysis, three subjects did not provide sufficient saliva volume for nighttime analysis, and one subject for morning analysis. One subject was determined to be an outlier using the modified Thompson tau test based on very low light exposure and excluded in regression analyses. For the remaining subjects, baseline morning melatonin, but not nighttime melatonin, was statistically significantly associated with time spent outdoors, total daily cumulative broad band light exposure (Figure 3-9), blue light exposure, and red light exposure ($p < 0.05$ for all, see Table 3-3 for statistics).

Nighttime melatonin at baseline was 16.1 ± 7.5 pg/mL (Figure 3-10). After the 2 week experimental period, nighttime melatonin statistically significantly increased 58% to 25.5 ± 10.7 pg/mL (effect size $r = -0.44$, t stat = -3.95, df = 19, $p = 0.0005$). Morning

melatonin decreased from 5.3 ± 2.8 pg/mL to 4.5 ± 2.6 pg/mL (not significantly different, $p = 0.16$).

PSQI scores decreased (i.e. improved) or remained the same for all subjects after the experimental period, with an average score of 5.6 ± 2.9 at baseline, and a score of 3.0 ± 2.2 after wearing blue blocking glasses (effect size $r = 0.43$, t stat = 7.23, $df = 20$, $p < 0.0005$). Objectively measured sleep duration statistically significantly increased by 24 minutes, from 408.7 ± 44.9 minutes to 431.5 ± 42.9 minutes (effect size $r = -0.25$, t stat = -3.77, $df = 20$, $p = 0.001$). Average baseline time of sleep was 12:24 am ± 1:04, and during the experimental period was 11:57 pm ± 1:03, which was statistically significantly earlier by 27 minutes (effect size $r = 0.22$, t stat = 3.33, $df = 19$, $p = 0.004$). Average morning wake time was similar between baseline, 7:18 am ± 0:38, and during the experimental period, 7:12 am ± 0:44 ($p = 0.34$). Sleep efficiency was not significantly different between baseline, 83.0 ± 9.2%, and the experimental period, 84.0 ± 6.1% ($p = 0.37$). Sleep latency was not significantly different between baseline, 12.4 ± 8.7 min, and the experimental period, 16.3 ± 10.6 min ($p = 0.12$). Mean daily activity in counts per minute was 260 ± 64 during the baseline week, and 266 ± 65 during the experimental period ($p = 0.42$).

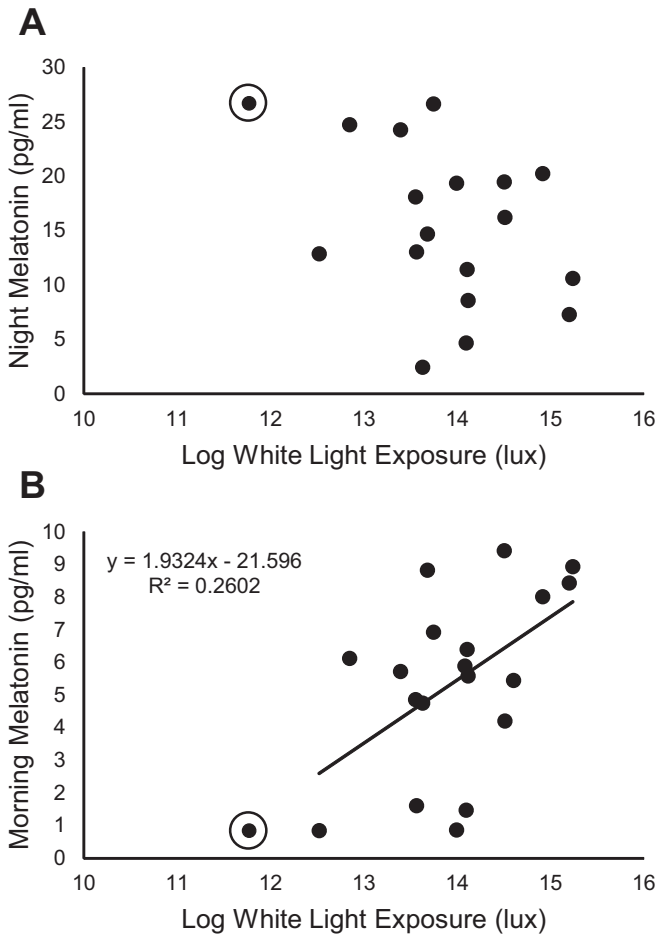

Figure 3-9. For the baseline week, (a) night melatonin was not significantly associated with total daily log white light exposure (P = 0.32). (b) Morning melatonin levels were statistically significantly associated with total daily log white light exposure during the baseline week (P < 0.05). Circled points are outliers and were not included in regression analyses.

Table 3-3. Linear regression statistics for morning melatonin and objectively measured time outdoors, and total daily broadband light exposure, and short and long wavelength light exposure.

	R^2	df	F	p-value
Time outdoors	0.25	19	5.85	0.02
Broad band light exposure	0.26	19	8.97	0.008
Short wavelength (blue) light exposure	0.34	19	9.46	0.007
Long wavelength (red) light exposure	0.30	19	7.89	0.01

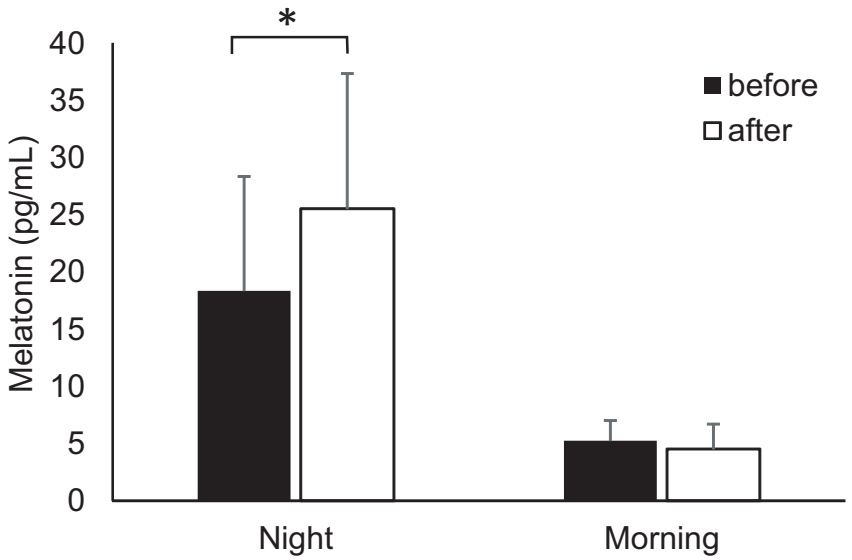

Figure 3-10. Melatonin levels measured via salivary assay at night and in the morning before (solid bars) and after (open bars) wearing blue blocking glasses at night-time for 2 weeks. Error bars denote standard deviation. * indicates $P < 0.05$

Pupil analysis

Resting pupil diameter at baseline following dark adaptation was 6.01 ± 0.86 mm. After the experimental period, resting dark adapted pupil diameter was 6.18 ± 1.1 mm (not significantly different, $p = 0.65$). Baseline resting pupil diameter was statistically significantly correlated with age ($R^2 = 0.34$, $df = 20$, $F = 9.85$, $p = 0.005$). Minimum pupil diameter during 1 s and 5 s short wavelength light stimulations at baseline were 2.87 ± 0.6 mm and 2.18 ± 0.39 mm, respectively. This increased, but was not statistically different, following the experimental period to 3.05 ± 0.76 mm and 2.32 ± 0.69 mm ($p = 0.41$ and 0.28).

Several parameters of the pupil response showed statistically significant changes following the experimental period (Table 3-4). Figure 3-11 shows the mean pupil traces for all subjects to 1 s long and short wavelength stimuli before and after the experimental period, illustrating a delayed redilation period following short wavelength stimulus offset after the experimental period. The 30 s PIPR to a 1 s short wavelength stimulus increased and the AUC for 1 s and 5 s short wavelength stimuli decreased, suggesting increased ipRGC activity (Figure 3-12). Observed increases in the 6 s PIPR following the experimental period did not reach statistical significance. There were no significant differences in any pupil metrics to long wavelength light before and after the experimental period.

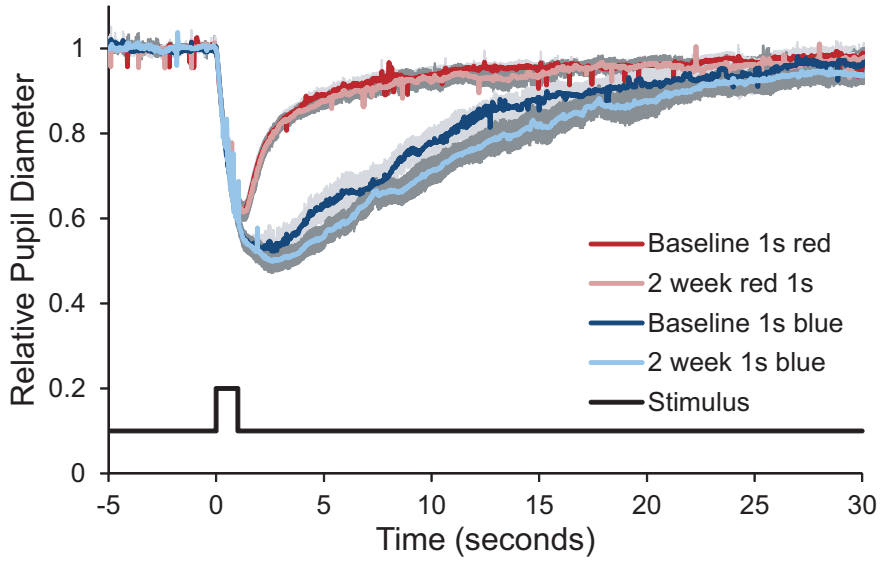

Figure 3-11. Mean normalized pupil diameter for all subjects (n = 21) during a 1 s long wavelength and a 1 s short wavelength stimulus at baseline (dark red and blue, respectively) and after wearing blue blocking glasses at nighttime for 2 weeks (light red and blue, respectively). 95% confidence intervals are shown in light gray for mean baseline pupil diameter and dark gray for 2 week mean pupil diameter.

Table 3-4. Statistical analysis for changes in pupil metrics to a 1 s and 5 s short wavelength stimulus before and after the experimental period. * indicates $p < 0.05$

Stimulus	PIPR metric	Effect size r	df	t stat	p
1 s	6 s PIPR	0.22	19	1.68	0.06
	30 s PIPR	0.31	19	3.17	* 0.005
	AUC	0.34	19	3.68	* 0.002
5 s	6 s PIPR	0.09	20	-0.08	0.53
	30 s PIPR	-0.04	20	-0.94	0.82
	AUC	0.23	20	2.51	* 0.02

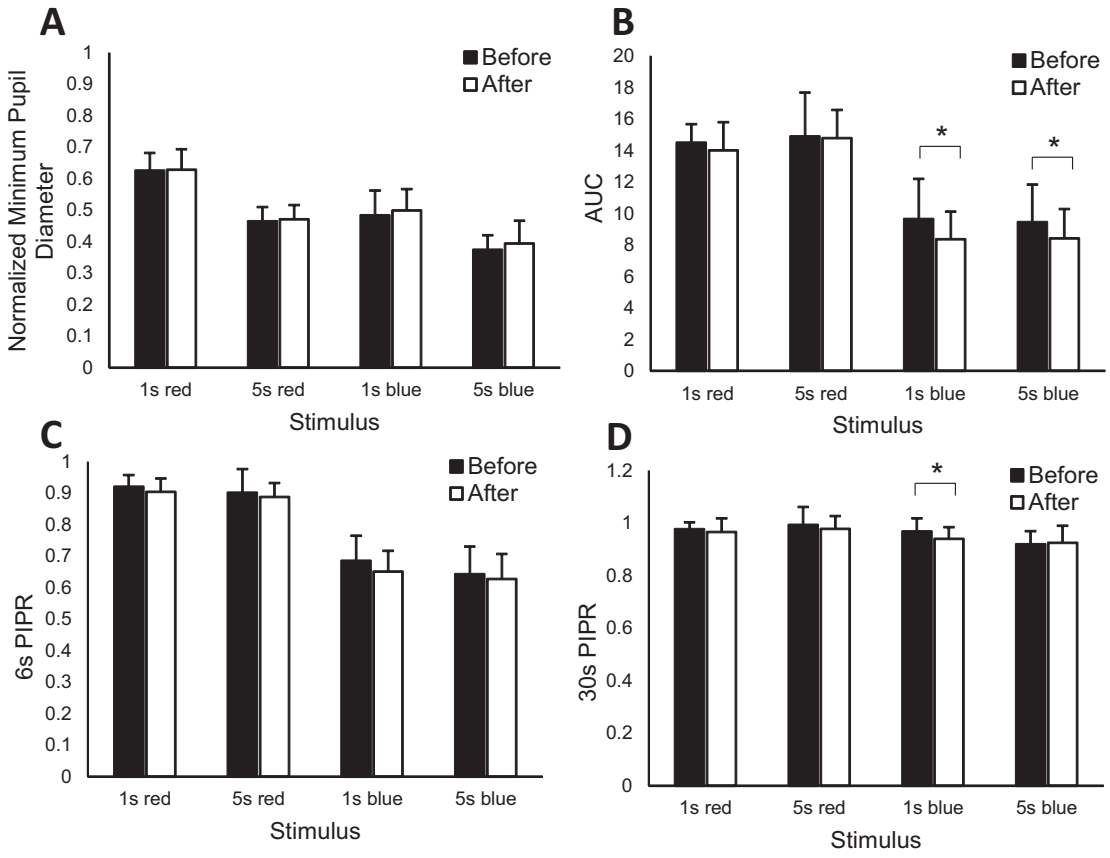

Figure 3-12. PIPR metrics to 1 s and 5 s long wavelength (red) and short wavelength (blue) stimuli before (solid bars) and after (open bars) wearing blue blocking glasses at nighttime for 2 weeks. A) normalized minimum pupil diameter, B) area under the curve (AUC), C) 6 s PIPR, D) 30 s PIPR. Error bars denote standard deviation.
* indicates $p < 0.05$

3.4 Discussion

This study demonstrates that the PIPR, which is driven primarily by direct activation of melanopsin in the ipRGCs, is subject to modulation by attenuating short wavelength input at night. The ipRGC-driven pupil response has been shown to undergo circadian variations.[78] Zele et al., showed that the PIPR increases throughout the morning, with increased redilation dynamics towards the peak at 14:58 hours. Our results suggest that by attenuating short wavelength light approximately four hours prior to subjects' habitual bedtime, induced either an advance in the phase of circadian rhythm, or an increase in sensitivity of the ipRGCs, so that some metrics of the PIPR, measured at the same time of day as baseline measures, increased following the experimental period compared to the baseline period. Based on observed changes in sleep parameters and increase in nighttime melatonin, we speculate that circadian phase was advanced. The blue blocking glasses likely prevented the circadian delay that has been shown to occur with exposure to light in the evening.[252]

Blocking artificial short wavelength light at nighttime resulted in a statistically significant subjective improvement in sleep quality and an objective increase in sleep duration of 24 minutes per night. Subjects went to sleep 27 minutes earlier during the experimental period compared to baseline. Additionally, the PSQI score improved from poor sleep quality to good sleep quality. While no subjects had diagnosed sleep abnormalities at baseline, the mean PSQI score of 5.6 indicates that some subjects did have poor quality sleep at baseline, which improved following the experimental period. Melatonin levels, as measured through a salivary assay, statistically significantly increased 58% at nighttime following approximately 4 hours of blue blocking glasses

wear (from approximately sunset to bedtime) for 2 weeks. Altered pupil dynamics following the experimental period suggest that demonstrated improvements in sleep and increases in melatonin are potentially mediated by the ipRGCs.

We showed statistically significant changes in the 30 s PIPR as well as the AUC following 2 weeks of blocking short wavelength light at nighttime. An increase in the 6 s PIPR was observed, but the change did not reach statistical significance. Some studies have suggested that the 6 s PIPR is the most sensitive metric, at least in terms of intra- and inter-individual variation.[204] Perhaps with a larger sample size, observed changes in the 6 s PIPR would have reached statistical significance. The increase in 30 s PIPR and decrease in AUC suggest that the firing of ipRGCs following light offset is potentially sustained longer following decreased nighttime input. Alternatively, there could be modifications in signal gain of the pupil control pathway. The change in measured activity could be due to a shift in the diurnal variation previously demonstrated in the PIPR.[78] In a similar study in which subjects with delayed sleep phase disorder wore blue light-blocking glasses from 9:00 pm to bedtime, dim light melatonin onset was shown to advance by 78 minutes.[253] While the previous study did not measure pupil responses, circadian shifts in melatonin suggest that the ipRGCs may also be undergoing circadian changes, as the ipRGC pathway ultimately leads to the pineal gland to control the release of melatonin. The increase in PIPR seen here suggests that a clinically significant change was induced in the ipRGCs as seen by the subsequent downstream increase in nighttime melatonin and increase in objectively measured sleep duration.

Previous studies have evaluated circadian photoentrainment and sleep following implantation of blue-blocking intraocular lenses (IOLs).[254, 255] The authors reported that

patients with blue-blocking IOLs showed no differences in any ipRGC-driven pupil responses, sleep-specific Actigraph measures, melatonin onset or PSQI scores compared to subjects with neutral IOLs after one year. The authors did find that peak melatonin concentration was 50% lower in the blue-blocking IOL group compared to the neutral IOL group. The study design of the IOL studies is very different than that of the current study, in that IOL subjects were viewing blue blocking conditions at all times, i.e. the subjects never had the opportunity to view full intensity blue light in the environment. The number of hours of light and dark were not altered, whereas here, blue-blocking lenses were only used at a particular time of the day, which increased the number of hours that blue light was not available, while not eliminating blue light at all hours of the day. By increasing the hours without blue light stimulation, "darkness" was effectively shifted earlier in the evening, resulting in alterations to nighttime melatonin concentration, increases in sleep duration and an increase in the PIPR as measured in the morning.

Here, the Actiwatch Spectrum was employed to measure subjects' habitual sleep and light exposure patterns over a three week period. The Actiwatch Spectrum is ideal because in addition to continuous activity and sleep measures, ambient illumination is recorded in terms of broad band light exposure and spectral composition. Modern sensor technology now allows these types of sleep studies to be carried out in subjects' homes,[256, 257] in some cases, replacing the need for overnight stays in sleep labs.[241, 258, 259] The Actiwatch has been shown to be similar to polysomnography, the gold standard for monitoring sleep,[260] and has been actively used in sleep related studies.[261, 262] Specifically, the Actiwatch-derived sleep duration was demonstrated to be strongly

correlated to that derived from polysomnography.[260] Actigraphy has been shown to be a reliable and cost-effective method to obtain continuous measurements of sleep and activity.[257] However, home monitoring does present some limitations. Subjects were instructed to go about their daily routine, without a specification of when they should go to bed or wake up, or how to set their environmental illumination, which may have introduced variability to the data that could have been controlled within a sleep facility. We also utilized the Actiwatch for measuring light exposure.[202] In order to measure light exposure, the device must be unobstructed by clothing. If the data indicated that the device was obstructed, the data were not included in the analysis. Additionally, the device is located at wrist level as opposed to eye level, which could potentially result in a discrepancy between measured and actual light exposure. However, the correlation between light exposure measured at eye level versus the wrist has been reported to be 0.76.[263]

Intrinsic stimulation of ipRGCs is most sensitive to short wavelength light with a peak at ~482 nm, although the spectral sensitivity spans a wider range.[10] The ipRGCs also receive input from rod and cone photoreceptors.[26, 32, 264] The majority of direct input to the ipRGCs was blocked via short wavelength absorbing glasses; however, the ipRGCs continued to receive synaptic (extrinsic) stimulation from long wavelength light via the rod and cone pathway. Unless subjects are in complete darkness, it is not possible to block all input to the ipRGCs. However, input from the rod-cone circuitry does not activate melanopsin, and these extrinsic responses have different temporal properties than intrinsic signals,[29] suggesting that demonstrated changes in dynamics were a function of successfully reducing melanopsin-driven ipRGC signaling at nighttime.

In this study, a control group with clear lenses was not utilized, potentially introducing a placebo effect. The blue blocking glasses used here (Uvex) are yellow tinted, and therefore, it would have been difficult to blind subjects as to which condition they were in. As opposed to many narrow spectrum blue blocking lenses that appear clear, the Uvex lenses block 99% of blue light, and the goal of this experiment was to decrease short wavelength stimulation to the highest degree possible. To minimize a placebo effect, we sought to use objective measures of sleep, melatonin and the pupil at baseline for each subject as a control. Additionally, subjects were not made aware of the changes we expected to observe in sleep following glasses wear. The increase in nighttime melatonin and increased sleep duration afforded from the Uvex glasses was robust, and it is possible that these increases would remain if lenses that decrease, rather than block, blue light are utilized. Therefore, to expand on the results found here, a randomized controlled experiment could be performed in the future in which clear lenses are utilized and subjects are blinded to whether the lenses filter blue light or not.

A potential limitation for the use of orange tinted glasses before bedtime as a therapeutic method to improve sleep is the yellow tinted percept induced by the lenses. Subjects reported that they adapted to the yellow tint after wearing the glasses for about ten minutes. However, the tint may cause concern during some activities such as night driving. Additionally, some individuals might be self-conscious about wearing the lenses outside of the home. These limitations could be combatted in the future through the development of clear blue blocking lenses that can block close to 100% of the short wavelength light. Moreover, it may not be necessary to wear the glasses every night to appreciate beneficial effects.

In conclusion, attenuating input to the ipRGCs via short wavelength-blocking glasses at night is a practical method to increase endogenous melatonin before sleep, improve sleep duration and help regulate circadian rhythm by combatting the abundance of nighttime blue light exposure, while allowing the continued use of artificial light and electronic devices after sunset. Evidence suggests that these demonstrated improvements in sleep are mediated by melanopsin-driven ipRGC activity.

3.5 Acknowledgements

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Chapter Four

Discussion

The first study investigated potential relationships between the ipRGC-driven pupil response, light exposure, and refractive error as well as downstream relationships between light exposure, sleep metrics, and melatonin in myopic and emmetropic individuals. Increased time outdoors during the previous week was associated with higher morning melatonin concentration. Morning melatonin concentration was significantly associated with white light exposure over the previous 7 days, but not the previous 1, 3, or 5 days. Similarly, morning melatonin concentration was also associated with red and blue light exposure over the previous 7 days. Myopes reported poorer sleep quality than emmetropic subjects indicated through the PSQI ($P = 0.036$). Scores 5 or greater indicate poor sleep quality, and emmetropic subjects reported good sleep quality on average (4.2 ± 2.3) while myopic subjects reported poor sleep quality on average (5.6 ± 2.2) with the overall average PSQI being 5.2 ± 2.7. There was no significant difference in sleep quality between subjects with low myopia (> -0.50 to -4.75) and high myopia (> -4.75). For the subset of subjects that wore an Actiwatch for objective measures of sleep prior to ipRGC measurement, mean daily sleep duration between refractive error groups somewhat trended towards emmetropes having a greater nightly sleep duration with emmetropes sleeping 457.2 ± 38.1 minutes per night, and myopes sleeping 421.7 ± 43.1 minutes per night, but this difference did not reach statistical significance ($P = 0.09$). Sleep efficiency was not statistically significant between refractive error groups. Trends suggested that the 6 s PIPR to 1 s short wavelength stimulation was associated with white light exposure over the previous day ($P = 0.05$) and blue light exposure over the previous day ($P = 0.03$). However, these relationships were not significant following Bonferroni correction to a significance level of $p \leq 0.006$. There was no relationships found between any

subjectively or objectively measured light exposure, over any length of time, in any particular wavelength with any PIPR metric and light stimulus. Additionally, no significant differences in pupil metrics were found between refractive error groups after Bonferonni correction.

Our second study investigated if alleviation of short-wavelength light exposure in the evening hours would result in alterations to sleep and pupil responses, such as melatonin levels, sleep metrics, and PIPR. Nightime melatonin was significantly higher during the experimental period than during the baseline period, while morning melatonin, though not significant ($p=0.16$), showed tendencies to be lower in the morning after the experimental period when compared to the baseline period. Nighttime melatonin increased 58% on average from 16.1 ± 7.5 pg/mL to 25.5 ± 10.7 pg/mL. PSQI scores improved (decreased) or remained the same for all subjects with an average score of 5.6 ± 2.9 at baseline and 3.0 ± 2.2 after the experimental period. Sleep duration, measured objectively through the Actiwatch, increased on average 24 minutes, from 408.7 ± 44.9 minutes to 431.5 ± 42.9 minutes. Additional sleep was gained through earlier bedtimes, where sleep onset averaged 27 minutes earlier during the experimental period, from 12:24 am ± 1:04 to 11:57 pm ± 1:03. Average morning wake time was not significantly different, 7:18 am ± 0:38 during the baseline week and 7:12 am ± 0:44 during the experimental weeks. Sleep efficiency, sleep latency, time outdoors, daily light exposure, and activity levels were similar between the baseline and experimental periods. Suggestive of increased ipRGC activity following blue-blocking glasses wear, the 30 s PIPR to a 1 s short wavelength stimulus increased and the AUC for 1 s and 5 s short wavelength stimuli decreased. Differences in PIPR were only found with short-

wavelength light, which directly stimulates ipRGCs, and not for long-wavelength light. As expected, baseline pupil diameter was significantly correlated with age. Resting pupil diameter was not significantly different between baseline and experimental periods. Our findings suggest that by decreasing nighttime input to the ipRGCs, the circadian rhythm shifted and therefore, sensitivity was altered when measured at the same time of day before and after the experimental period.

Despite not reaching statistical significance, our results displayed trends that increased light exposure over the previous day was associated with the amplitude of the PIPR to a 1 s short wavelength light stimulus, as measured at one consistent time point in the morning, suggesting increased morning ipRGC activity, or a shift in circadian phase, with increased habitual light exposure. This trend is in accordance with a previous study that showed slowed pupillary redilation (i.e. increased ipRGC activity) to 0.1 Hz flashing stimuli with greater habitual light exposure.[200]

The most likely explanation for the changes observed in melatonin levels as well as PIPR is the inhibition of circadian delay which has been show to occur when individuals are exposed to light in the evening.[252] A similar study demonstrated the advance of dim light melatonin onset by 78 minutes through the wearing of blue-blocking glasses from 9:00pm until bedtime.[253] Previous studies have found similar results demonstrating that wearing of blue-blocking glasses in the evening can lead to increased nighttime melatonin and improvements in sleep quality.[165] [166] Our study was the first to demonstrate the alteration of melatonin levels with a clinically significant increase in PIPR suggesting that ipRGCs were responsible for the downstream increase in nighttime melatonin and increase in objectively measured sleep duration. By alleviating blue light

stimulation in the evening hours, perceived nighttime was effectively shifted earlier in the evening, resulting in alterations to nighttime melatonin concentrations, increases in sleep duration, and an increase in the PIPR as measured in the morning. Similar to the results in our second study, a recent study reported higher morning melatonin concentrations in the elderly with increased daytime light exposure.[207] Despite our average age being much younger, the methodology in this study was similar.

Previous studies have conflicting results as to whether PIPR is associated age. With normalization to baseline pupil size, Zele, et al, found PIPR to be independent of age, while Herbst, et al, suggested some ipRGC driven pupil response metrics may be associated with age, presumably due to increased light scatter from the lens in older patients. [86, 90] Our population was limited to subjects under the age of 40, but our results were consistent with the prior study in which PIPR was found to be independent of age.

Despite not reaching statistical significance, trends for higher morning melatonin levels in myopic subjects were observed, which is similar to a recent study.[191] This could partially explain our finding that individuals with myopia reported poorer sleep quality, potentially due to a delayed circadian cycle where melatonin levels peak later in the evening thus resulting in higher morning melatonin levels.

This research demonstrates the remarkable capability to manipulate circadian rhythm by altering melatonin levels through diminishing short-wavelength light exposure. Implications for such ability could prove to be useful in a wide array of sleep disorders and circadian rhythm dysfunctions. One application is through the use of blue-blocking tints in the evening hours to improve nighttime sleep duration and quality. Such lenses offer a more potent and effective circadian rhythm modulator and, in the long-

term, a cheaper option than melatonin supplements.[265] Melatonin modulation could also prove useful in cases of nightshift workers whose ambient light cues the biological clock to a different sleep cycle than needed or people traveling abroad who must adjust to a new light cycle. With the increase in use of LED screens on electronic devices, which largely emit blue light, sleep dysfunction is becoming more prevalent and blue-blocking tints are a tool to combat such a problem.

This research also is a step forward in the understanding of environmental factors affecting refractive error and eye growth. With myopia rapidly becoming a societal and ocular health concern, modifiable life factors contributing to axial elongation are of increasing importance. While genetics are uncontrollable, environmental cues such as light exposure are modifiable and known to affect both eye growth and circadian rhythm. This research also began to explore relationships between refractive error, sleep quality, and melatonin levels. Hormones and neurotransmitters affected by environmental factors could partially explain development of refractive error through eye growth. Some potential explanations for the effect of light exposure on axial elongation include, dopamine or melatonin levels, sun-derived vitamin D levels, or even changes in optical aberrations with pupillary constriction. However, much more research is necessary to understand any potential relationships.

In our studies, sleep and light exposure were not controlled for, but measured objectively using wearable sensors in the subject's habitual environment. Home monitoring of sleep metrics presents challenges in accurate data collection. While a major advantage includes the subject continuing their habitual routines, controlling for lifestyle variability and the most consistent conditions among subjects would have been

conducting the entire experiment in a sleep facility. Use of an amber tint in the blue-blocking glasses alters color and visual perception of the subjects, therefore not allowing for a randomized control and potentially allowing for a placebo affect, although subjects were not told of the expected changes to circadian rhythm. An ideal study would include two sets of clear glasses, one with blue-blocking capability and the other with a placebo. While subjects reported excellent compliance with the wearing of the blue-blocking glasses, it is conceivable that some subjects may have disregarded wear in social settings due to cosmetic concerns.

In both studies, an Actiwatch was utilized in the collection of light exposure data. This device being worn at wrist level is somewhat limited in the determination of light exposure at eye level. Ideally, a device would allow for measurements of light exposure to be collected at eye level. Also, in both studies additional melatonin sample collections throughout a 24 hour period would have been beneficial. In the first study melatonin was only collected at one time point. The second study included four total collections; two morning and two evening collections, one set in the control week and the other after wearing blue-blocking glasses for two weeks.

Future research directions should include a blind and randomized controlled experiment where clear lenses are utilized to mask which subjects are using blue-blocking lenses versus non-absorbing lenses. Additionally, research could begin to be performed examining the use of alleviating short wavelength light exposure at specific times to readapt circadian rhythms for nightshift workers or even for intercontinental flights. Our first project should be expanded to children to investigate relationships between melatonin, dopamine, axial elongation, refractive error, activity levels, and light

exposure separated by wavelength. An additional study of melatonin, sleep metrics, time outdoors, light exposures comparing not only emmetropes and myopes, but also hyperopes would be beneficial as our study did recruit a sufficient amount of hyperopes to obtain this data.

In conclusion, ipRGCs possess the capability of remarkable visual and non-visual forming roles, which may be susceptible to manipulation through attenuation of specific wavelengths thus resulting in behavioral and physiological modulation. Similarly, light exposure of varying wavelength, duration, and intensity may influence circadian rhythms through melatonin release modulated by ipRGCs. There also exists a complex relationship between light exposure, ipRGCs, sleep, and refractive error that may someday contribute to the understanding of how modifiable environmental factors influence refractive error development and axial elongation.

References

1. Keeler CE, Sutcliffe E, Chaffee EL. Normal and "Rodless" Retinae of the House Mouse with Respect to the Electromotive Force Generated through Stimulation by Light. Proc Natl Acad Sci U S A. 1928;14(6):477-84.
2. C.E. K. Iris movements in blind mice. Am J Physiol1927. pp. 107-12.
3. Freedman MS, Lucas RJ, Soni B, von Schantz M, Muñoz M, David-Gray Z, et al. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science. 1999;284(5413):502-4.
4. Lucas RJ, Freedman MS, Muñoz M, Garcia-Fernández JM, Foster RG. Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. Science. 1999;284(5413):505-7.
5. Klein DC, Weller JL. Rapid light-induced decrease in pineal serotonin N-acetyltransferase activity. Science. 1972;177(4048):532-3.
6. Zaidi FH, Hull JT, Peirson SN, Wulff K, Aeschbach D, Gooley JJ, et al. Short-wavelength light sensitivity of circadian, pupillary, and visual awareness in humans lacking an outer retina. Curr Biol. 2007;17(24):2122-8.
7. Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: An opsin in melanophores, brain, and eye. Proc Natl Acad Sci U S A. 1998;95(1):340-5. Epub 1998/02/21.
8. Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. J Neurosci. 2000;20(2):600-5.
9. Provencio I, Rollag MD, Castrucci AM. Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. Nature. 2002;415(6871):493.
10. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. Science. 2002;295(5557):1070-3.
11. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science. 2002;295(5557):1065-70. Epub 2002/02/09.
12. Lucas RJ, Douglas RH, Foster RG. Characterization of an ocular photopigment capable of driving pupillary constriction in mice. Nat Neurosci. 2001;4(6):621-6.
13. Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, Yau KW. Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science. 2003;299(5604):245-7. Epub 2003/01/11.
14. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. J Comp Neurol. 1990;292(4):497-523.
15. Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature. 2005;433(7027):749-54. Epub 2005/02/18.
16. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. J Comp Neurol. 2006;497(3):326-49. Epub 2006/06/01.
17. Morin LP, Blanchard JH, Provencio I. Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: bifurcation

and melanopsin immunoreactivity. J Comp Neurol. 2003;465(3):401-16. Epub 2003/09/11.
18. Paul KN, Saafir TB, Tosini G. The role of retinal photoreceptors in the regulation of circadian rhythms. Rev Endocr Metab Disord. 2009;10(4):271-8.
19. LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci. 2014;15(7):443-54. Epub 2014/06/11.
20. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. Nat Neurosci. 2001;4(12):1165. Epub 2001/11/20.
21. Hannibal J, Hindersson P, Knudsen SM, Georg B, Fahrenkrug J. The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. J Neurosci. 2002;22(1):RC191. Epub 2002/01/05.
22. Gooley JJ, Lu J, Fischer D, Saper CB. A broad role for melanopsin in nonvisual photoreception. J Neurosci. 2003;23(18):7093-106. Epub 2003/08/09.
23. Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, et al. Photon capture and signalling by melanopsin retinal ganglion cells. Nature. 2009;457(7227):281-7. Epub 2009/01/02.
24. Brown TM, Gias C, Hatori M, Keding SR, Semo M, Coffey PJ, et al. Melanopsin contributions to irradiance coding in the thalamo-cortical visual system. PLoS Biol.8(12):e1000558. Epub 2010/12/15.
25. Schmidt TM, Chen SK, Hattar S. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. Trends Neurosci. 2011;34(11):572-80. Epub 2011/08/03.
26. Guler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature. 2008;453(7191):102-5. Epub 2008/04/25.
27. Belenky MA, Smeraski CA, Provencio I, Sollars PJ, Pickard GE. Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. J Comp Neurol. 2003;460(3):380-93. Epub 2003/04/15.
28. Perez-Leon J, Warren E, Allen C, Robinson D, Brown R. Synaptic inputs to retinal ganglion cells that set the circadian clock. European Journal of Neuroscience2006

29. Wong KY, Dunn FA, Graham DM, Berson DM. Synaptic influences on rat ganglion-cell photoreceptors. J Physiol. 2007;582(Pt 1):279-96. Epub 2007/05/19.
30. Zhao X, Stafford BK, Godin AL, King WM, Wong KY. Photoresponse diversity among the five types of intrinsically photosensitive retinal ganglion cells. J Physiol.592(Pt 7):1619-36. Epub 2014/01/08.
31. Weng S, Estevez ME, Berson DM. Mouse ganglion-cell photoreceptors are driven by the most sensitive rod pathway and by both types of cones. PLoS One. 2013;8(6):e66480. Epub 2013/06/07.
32. Jusuf PR, Lee SC, Hannibal J, Grunert U. Characterization and synaptic connectivity of melanopsin-containing ganglion cells in the primate retina. Eur J Neurosci. 2007;26(10):2906-21. Epub 2007/11/16.
33. Schmidt TM, Kofuji P. Differential cone pathway influence on intrinsically photosensitive retinal ganglion cell subtypes. J Neurosci.30(48):16262-71. Epub 2010/12/03.

34. Vugler AA, Redgrave P, Semo M, Lawrence J, Greenwood J, Coffey PJ. Dopamine neurones form a discrete plexus with melanopsin cells in normal and degenerating retina. Exp Neurol. 2007;205(1):26-35. Epub 2007/03/17.
35. Van Hook MJ, Wong KY, Berson DM. Dopaminergic modulation of ganglion-cell photoreceptors in rat. Eur J Neurosci.35(4):507-18. Epub 2012/02/07.
36. Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG. Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. Proc Natl Acad Sci U S A. 2008;105(37):14181-6. Epub 2008/09/08.
37. Isoldi MC, Rollag MD, Castrucci AM, Provencio I. Rhabdomeric phototransduction initiated by the vertebrate photopigment melanopsin. Proc Natl Acad Sci U S A. 2005;102(4):1217-21. Epub 2005/01/18.
38. Hannibal J, Georg B, Hindersson P, Fahrenkrug J. Light and darkness regulate melanopsin in the retinal ganglion cells of the albino Wistar rat. J Mol Neurosci. 2005;27(2):147-55. Epub 2005/09/28.
39. Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, et al. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. Science. 2002;298(5601):2213-6. Epub 2002/12/14.
40. Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, et al. Melanopsin is required for non-image-forming photic responses in blind mice. Science. 2003;301(5632):525-7. Epub 2003/06/28.
41. Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, et al. Role of melanopsin in circadian responses to light. Science. 2002;298(5601):2211-3. Epub 2002/12/14.
42. Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, et al. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature. 2003;424(6944):76-81. Epub 2003/06/17.
43. Melyan Z, Tarttelin EE, Bellingham J, Lucas RJ, Hankins MW. Addition of human melanopsin renders mammalian cells photoresponsive. Nature. 2005;433(7027):741-5. Epub 2005/01/28.
44. Panda S, Nayak SK, Campo B, Walker JR, Hogenesch JB, Jegla T. Illumination of the melanopsin signaling pathway. Science. 2005;307(5709):600-4. Epub 2005/02/01.
45. Qiu X, Kumbalasiri T, Carlson SM, Wong KY, Krishna V, Provencio I, et al. Induction of photosensitivity by heterologous expression of melanopsin. Nature. 2005;433(7027):745-9. Epub 2005/01/28.
46. Mure LS, Cornut PL, Rieux C, Drouyer E, Denis P, Gronfier C, et al. Melanopsin bistability: a fly's eye technology in the human retina. PLoS One. 2009;4(6):e5991. Epub 2009/06/25.
47. Sexton TJ, Golczak M, Palczewski K, Van Gelder RN. Melanopsin is highly resistant to light and chemical bleaching in vivo. J Biol Chem. 2012;287(25):20888-97. Epub 2012/04/30.
48. Sand A, Schmidt TM, Kofuji P. Diverse types of ganglion cell photoreceptors in the mammalian retina. Prog Retin Eye Res. 2012;31(4):287-302. Epub 2012/03/26.
49. Joo HR, Peterson BB, Dacey DM, Hattar S, Chen SK. Recurrent axon collaterals of intrinsically photosensitive retinal ganglion cells. Vis Neurosci.30(4):175-82. Epub 2013/07/10.

50. Liao HW, Ren X, Peterson BB, Marshak DW, Yau KW, Gamlin PD, et al. Melanopsin-expressing ganglion cells on macaque and human retinas form two morphologically distinct populations. J Comp Neurol. 2016;524(14):2845-72.
51. Nasir-Ahmad S, Lee SC, Martin PR, Grunert U. Melanopsin-expressing ganglion cells in human retina: Morphology, distribution, and synaptic connections. J Comp Neurol. 2017.
52. Hannibal J, Christiansen AT, Heegaard S, Fahrenkrug J, Kiilgaard JF. Melanopsin expressing human retinal ganglion cells: Subtypes, distribution, and intraretinal connectivity. J Comp Neurol. 2017.
53. Hu C, Hill DD, Wong KY. Intrinsic physiological properties of the five types of mouse ganglion-cell photoreceptors. J Neurophysiol.109(7):1876-89. Epub 2013/01/25.
54. Baver SB, Pickard GE, Sollars PJ. Two types of melanopsin retinal ganglion cell differentially innervate the hypothalamic suprachiasmatic nucleus and the olivary pretectal nucleus. Eur J Neurosci. 2008;27(7):1763-70. Epub 2008/03/29.
55. Berson DM, Castrucci AM, Provencio I. Morphology and mosaics of melanopsin-expressing retinal ganglion cell types in mice. J Comp Neurol. 2010;518(13):2405-22.
56. Viney TJ, Balint K, Hillier D, Siegert S, Boldogkoi Z, Enquist LW, et al. Local retinal circuits of melanopsin-containing ganglion cells identified by transsynaptic viral tracing. Curr Biol. 2007;17(11):981-8. Epub 2007/05/26.
57. Schmidt TM, Kofuji P. Functional and morphological differences among intrinsically photosensitive retinal ganglion cells. J Neurosci. 2009;29(2):476-82.
58. Gamlin PD, McDougal DH, Pokorny J, Smith VC, Yau KW, Dacey DM. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. Vision Res. 2007;47(7):946-54. Epub 2007/02/27.
59. Ecker JL, Dumitrescu ON, Wong KY, Alam NM, Chen SK, LeGates T, et al. Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. Neuron.67(1):49-60. Epub 2010/07/14.
60. Müller LP, Do MT, Yau KW, He S, Baldridge WH. Tracer coupling of intrinsically photosensitive retinal ganglion cells to amacrine cells in the mouse retina. J Comp Neurol. 2010;518(23):4813-24.
61. Wong KY. A retinal ganglion cell that can signal irradiance continuously for 10 hours. J Neurosci.32(33):11478-85. Epub 2012/08/17.
62. Ogden TE, Miller RF. Studies of the optic nerve of the rhesus monkey: nerve fiber spectrum and physiological properties. Vision Res. 1966;6(9):485-506.
63. Graham DM, Wong KY, Shapiro P, Frederick C, Pattabiraman K, Berson DM. Melanopsin ganglion cells use a membrane-associated rhabdomeric phototransduction cascade. J Neurophysiol. 2008;99(5):2522-32. Epub 2008/02/27.
64. Fu Y, Zhong H, Wang MH, Luo DG, Liao HW, Maeda H, et al. Intrinsically photosensitive retinal ganglion cells detect light with a vitamin A-based photopigment, melanopsin. Proc Natl Acad Sci U S A. 2005;102(29):10339-44. Epub 2005/07/15.
65. Hatori M, Le H, Vollmers C, Keding SR, Tanaka N, Buch T, et al. Inducible ablation of melanopsin-expressing retinal ganglion cells reveals their central role in non-image forming visual responses. PLoS One. 2008;3(6):e2451. Epub 2008/06/12.
66. Brown TM, Tsujimura S, Allen AE, Wynne J, Bedford R, Vickery G, et al. Melanopsin-based brightness discrimination in mice and humans. Curr Biol.22(12):1134-41. Epub 2012/05/29.

67. Barlow RB, Verrillo RT. Brightness sensation in a ganzfeld. Vision Res. 1976;16(11):1291-7.
68. Horiguchi H, Winawer J, Dougherty RF, Wandell BA. Human trichromacy revisited. Proc Natl Acad Sci U S A. 2013;110(3):E260-9. Epub 2012/12/19.
69. Schmidt TM, Alam NM, Chen S, Kofuji P, Li W, Prusky GT, et al. A role for melanopsin in alpha retinal ganglion cells and contrast detection. Neuron.82(4):781-8. Epub 2014/05/24.
70. Sekaran S, Lupi D, Jones SL, Sheely CJ, Hattar S, Yau KW, et al. Melanopsin-dependent photoreception provides earliest light detection in the mammalian retina. Curr Biol. 2005;15(12):1099-107. Epub 2005/06/21.
71. Fahrenkrug J, Nielsen HS, Hannibal J. Expression of melanopsin during development of the rat retina. Neuroreport. 2004;15(5):781-4. Epub 2004/04/10.
72. Tarttelin EE, Bellingham J, Bibb LC, Foster RG, Hankins MW, Gregory-Evans K, et al. Expression of opsin genes early in ocular development of humans and mice. Exp Eye Res. 2003;76(3):393-6.
73. Delwig A, Logan AM, Copenhagen DR, Ahn AH. Light evokes melanopsin-dependent vocalization and neural activation associated with aversive experience in neonatal mice. PLoS One. 2012;7(9):e43787. Epub 2012/09/13.
74. Johnson J, Wu V, Donovan M, Majumdar S, Rentería RC, Porco T, et al. Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A. 2010;107(40):17374-8. Epub 2010/09/20.
75. Rao S, Chun C, Fan J, Kofron JM, Yang MB, Hegde RS, et al. A direct and melanopsin-dependent fetal light response regulates mouse eye development. Nature.494(7436):243-6. Epub 2013/01/22.
76. McDougal DH, Gamlin PD. The influence of intrinsically-photosensitive retinal ganglion cells on the spectral sensitivity and response dynamics of the human pupillary light reflex. Vision Res. 2010;50(1):72-87.
77. Markwell EL, Feigl B, Zele AJ. Intrinsically photosensitive melanopsin retinal ganglion cell contributions to the pupillary light reflex and circadian rhythm. Clin Exp Optom.93(3):137-49. Epub 2010/06/19.
78. Zele AJ, Feigl B, Smith SS, Markwell EL. The circadian response of intrinsically photosensitive retinal ganglion cells. PloS one. 2011;6(3):e17860.
79. Park JC, Moura AL, Raza AS, Rhee DW, Kardon RH, Hood DC. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. Invest Ophthalmol Vis Sci. 2011;52(9):6624-35. Epub 2011/07/12.
80. Feigl B, Mattes D, Thomas R, Zele AJ. Intrinsically photosensitive (melanopsin) retinal ganglion cell function in glaucoma. Invest Ophthalmol Vis Sci.52(7):4362-7. Epub 2011/04/19.
81. Feigl B, Zele AJ, Fader SM, Howes AN, Hughes CE, Jones KA, et al. The post-illumination pupil response of melanopsin-expressing intrinsically photosensitive retinal ganglion cells in diabetes. Acta Ophthalmol.90(3):e230-4. Epub 2011/09/03.
82. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, Kawasaki A. Chromatic pupil responses: preferential activation of the melanopsin-mediated versus outer photoreceptor-mediated pupil light reflex. Ophthalmology. 2009;116(8):1564-73. Epub 2009/06/09.

83. Kankipati L, Girkin CA, Gamlin PD. Post-illumination pupil response in subjects without ocular disease. Invest Ophthalmol Vis Sci. 2010;51(5):2764-9. Epub 2009/12/10.
84. Kankipati L, Girkin CA, Gamlin PD. The post-illumination pupil response is reduced in glaucoma patients. Invest Ophthalmol Vis Sci.52(5):2287-92. Epub 2011/01/08.
85. Kawasaki A, Herbst K, Sander B, Milea D. Selective wavelength pupillometry in Leber hereditary optic neuropathy. Clin Exp Ophthalmol. 2010;38(3):322-4.
86. Herbst K, Sander B, Lund-Andersen H, Broendsted AE, Kessel L, Hansen MS, et al. Intrinsically photosensitive retinal ganglion cell function in relation to age: a pupillometric study in humans with special reference to the age-related optic properties of the lens. BMC Ophthalmol. 2012;12:4. Epub 2012/04/03.
87. Herbst K, Sander B, Milea D, Lund-Andersen H, Kawasaki A. Test-retest repeatability of the pupil light response to blue and red light stimuli in normal human eyes using a novel pupillometer. Front Neurol. 2011;2:10. Epub 2011/02/22.
88. Tsujimura S, Ukai K, Ohama D, Nuruki A, Yunokuchi K. Contribution of human melanopsin retinal ganglion cells to steady-state pupil responses. Proc Biol Sci.277(1693):2485-92. Epub 2010/04/09.
89. Munch M, Leon L, Crippa SV, Kawasaki A. Circadian and wake-dependent effects on the pupil light reflex in response to narrow-bandwidth light pulses. Invest Ophthalmol Vis Sci. 2012;53(8):4546-55.
90. Adhikari P, Pearson CA, Anderson AM, Zele AJ, Feigl B. Effect of age and refractive error on the melanopsin mediated post-illumination pupil response (PIPR). Sci Rep. 2015;5:17610.
91. Münch M, Léon L, Collomb S, Kawasaki A. Comparison of acute non-visual bright light responses in patients with optic nerve disease, glaucoma and healthy controls. Sci Rep. 2015;5:15185. Epub 2015/10/19.
92. Nissen C, Sander B, Milea D, Kolko M, Herbst K, Hamard P, et al. Monochromatic Pupillometry in Unilateral Glaucoma Discloses no Adaptive Changes Subserved by the ipRGCs. Front Neurol. 2014;5:15. Epub 2014/02/05.
93. Ishibashi F, Kojima R, Taniguchi M, Kosaka A, Uetake H, Tavakoli M. The Preferential Impairment of Pupil Constriction Stimulated by Blue Light in Patients with Type 2 Diabetes without Autonomic Neuropathy. J Diabetes Res. 2017;2017:6069730. Epub 2017/03/22.
94. Georg B, Ghelli A, Giordano C, Ross-Cisneros FN, Sadun AA, Carelli V, et al. Melanopsin-expressing retinal ganglion cells are resistant to cell injury, but not always. Mitochondrion. 2017. Epub 2017/04/12.
95. Perganta G, Barnard AR, Katti C, Vachtsevanos A, Douglas RH, MacLaren RE, et al. Non-image-forming light driven functions are preserved in a mouse model of autosomal dominant optic atrophy. PLoS One. 2013;8(2):e56350. Epub 2013/02/11.
96. Li RS, Chen BY, Tay DK, Chan HH, Pu ML, So KF. Melanopsin-expressing retinal ganglion cells are more injury-resistant in a chronic ocular hypertension model. Invest Ophthalmol Vis Sci. 2006;47(7):2951-8. Epub 2006/06/27.
97. Perez de Sevilla Muller L, Sargoy A, Rodriguez AR, Brecha NC. Melanopsin ganglion cells are the most resistant retinal ganglion cell type to axonal injury in the rat retina. PLoS One.9(3):e93274. Epub 2014/03/29.

98. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, Kawasaki A. Chromatic pupillometry in patients with retinitis pigmentosa. Ophthalmology. 2011;118(2):376-81.
99. Esquiva G, Lax P, Cuenca N. Impairment of intrinsically photosensitive retinal ganglion cells associated with late stages of retinal degeneration. Invest Ophthalmol Vis Sci.54(7):4605-18. Epub 2013/06/15.
100. Kawasaki A, Crippa SV, Kardon R, Leon L, Hamel C. Characterization of pupil responses to blue and red light stimuli in autosomal dominant retinitis pigmentosa due to NR2E3 mutation. Invest Ophthalmol Vis Sci. 2012;53(9):5562-9. Epub 2012/08/15.
101. Lorenz B, Strohmayr E, Zahn S, Friedburg C, Kramer M, Preising M, et al. Chromatic pupillometry dissects function of the three different light-sensitive retinal cell populations in RPE65 deficiency. Invest Ophthalmol Vis Sci.53(9):5641-52. Epub 2012/07/19.
102. Swann PG, Lovie-Kitchin JE. Age-related maculopathy. II: The nature of the central visual field loss. Ophthalmic Physiol Opt. 1991;11(1):59-70.
103. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. Invest Ophthalmol Vis Sci. 1996;37(7):1236-49.
104. Medeiros NE, Curcio CA. Preservation of ganglion cell layer neurons in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2001;42(3):795-803.
105. Feigl B, Zele AJ. Melanopsin-expressing intrinsically photosensitive retinal ganglion cells in retinal disease. Optom Vis Sci. 2014;91(8):894-903.
106. Schmid-Kubista KE, Glittenberg CG, Cezanne M, Holzmann K, Neumaier-Ammerer B, Binder S. Daytime levels of melatonin in patients with age-related macular degeneration. Acta Ophthalmol. 2009;87(1):89-93. Epub 2008/05/20.
107. Kawasaki A, Munier FL, Leon L, Kardon RH. Pupillometric quantification of residual rod and cone activity in leber congenital amaurosis. Arch Ophthalmol. 2012;130(6):798-800.
108. Atchison DA, Girgenti CC, Campbell GM, Dodds JP, Byrnes TM, Zele AJ. Influence of field size on pupil diameter under photopic and mesopic light levels. Clin Exp Optom. 2011;94(6):545-8. Epub 2011/09/20.
109. Feigl B. Age-related maculopathy - linking aetiology and pathophysiological changes to the ischaemia hypothesis. Prog Retin Eye Res. 2009;28(1):63-86. Epub 2008/12/03.
110. Göz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP. Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms. PLoS One. 2008;3(9):e3153. Epub 2008/09/05.
111. Larsen PJ, Enquist LW, Card JP. Characterization of the multisynaptic neuronal control of the rat pineal gland using viral transneuronal tracing. Eur J Neurosci. 1998;10(1):128-45.
112. Vartanian GV, Li BY, Chervenak AP, Walch OJ, Pack W, Ala-Laurila P, et al. Melatonin suppression by light in humans is more sensitive than previously reported. J Biol Rhythms.30(4):351-4. Epub 2015/05/29.
113. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. J Neurosci. 2001;21(16):6405-12.

114. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. J Physiol. 2001;535(Pt 1):261-7.
115. Czeisler CA, Shanahan TL, Klerman EB, Martens H, Brotman DJ, Emens JS, et al. Suppression of melatonin secretion in some blind patients by exposure to bright light. N Engl J Med. 1995;332(1):6-11.
116. Lockley SW, Dijk DJ, Kosti O, Skene DJ, Arendt J. Alertness, mood and performance rhythm disturbances associated with circadian sleep disorders in the blind. J Sleep Res. 2008;17(2):207-16.
117. Sack RL, Lewy AJ. Circadian rhythm sleep disorders: lessons from the blind. Sleep Med Rev. 2001;5(3):189-206.
118. Erren TC, Reiter RJ. Light Hygiene: Time to make preventive use of insights--old and new--into the nexus of the drug light, melatonin, clocks, chronodisruption and public health. Med Hypotheses. 2009;73(4):537-41.
119. Lupi D, Oster H, Thompson S, Foster RG. The acute light-induction of sleep is mediated by OPN4-based photoreception. Nat Neurosci. 2008;11(9):1068-73.
120. Tsai JW, Hannibal J, Hagiwara G, Colas D, Ruppert E, Ruby NF, et al. Melanopsin as a sleep modulator: circadian gating of the direct effects of light on sleep and altered sleep homeostasis in Opn4(-/-) mice. PLoS Biol. 2009;7(6):e1000125. Epub 2009/06/11.
121. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. Science. 1980;210(4475):1267-9.
122. SW L. Circadian Rhythms: Influence of Light in Humans. Encyclopedia of Neuroscience: Oxford Academic Press, 2009. pp. 971-88.
123. Lockley SW, Brainard GC, Czeisler CA. High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. J Clin Endocrinol Metab. 2003;88(9):4502-5.
124. Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 2005;90(3):1311-6. Epub 2004/12/07.
125. Revell VL, Skene DJ. Light-induced melatonin suppression in humans with polychromatic and monochromatic light. Chronobiol Int. 2007;24(6):1125-37.
126. Vandewalle G, Gais S, Schabus M, Balteau E, Carrier J, Darsaud A, et al. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure. Cereb Cortex. 2007;17(12):2788-95. Epub 2007/04/02.
127. Lockley SW, Evans EE, Scheer FA, Brainard GC, Czeisler CA, Aeschbach D. Short-wavelength sensitivity for the direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans. Sleep. 2006;29(2):161-8.
128. Munch M, Kobialka S, Steiner R, Oelhafen P, Wirz-Justice A, Cajochen C. Wavelength-dependent effects of evening light exposure on sleep architecture and sleep EEG power density in men. Am J Physiol Regul Integr Comp Physiol. 2006;290(5):R1421-8.
129. Revell VL, Arendt J, Fogg LF, Skene DJ. Alerting effects of light are sensitive to very short wavelengths. Neurosci Lett. 2006;399(1-2):96-100. Epub 2006/02/21.

130. Cajochen C, Jud C, Munch M, Kobialka S, Wirz-Justice A, Albrecht U. Evening exposure to blue light stimulates the expression of the clock gene PER2 in humans. Eur J Neurosci. 2006;23(4):1082-6.
131. Vandewalle G, Balteau E, Phillips C, Degueldre C, Moreau V, Sterpenich V, et al. Daytime light exposure dynamically enhances brain responses. Curr Biol. 2006;16(16):1616-21.
132. Vandewalle G, Schwartz S, Grandjean D, Wuillaume C, Balteau E, Degueldre C, et al. Spectral quality of light modulates emotional brain responses in humans. Proc Natl Acad Sci U S A. 2010;107(45):19549-54. Epub 2010/10/25.
133. Lynch HJ, Ozaki Y, Shakal D, Wurtman RJ. Melatonin excretion of man and rats: effect of time of day, sleep, pinealectomy and food consumption. Int J Biometeorol. 1975;19(4):267-79.
134. Jimerson DC, Lynch HJ, Post RM, Wurtman RJ, Bunney WE. Urinary melatonin rhythms during sleep deprivation in depressed patients and normals. Life Sci. 1977;20(9):1501-8.
135. Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM. Human melatonin production decreases with age. J Pineal Res. 1986;3(4):379-88.
136. Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron. 1994;13(5):1177-85.
137. Pang SF, Dubocovich ML, Brown GM. Melatonin receptors in peripheral tissues: a new area of melatonin research. Biol Signals. 1993;2(4):177-80.
138. Morgan PJ, Barrett P, Howell HE, Helliwell R. Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Int. 1994;24(2):101-46.
139. Stankov B, Reiter RJ. Melatonin receptors: current status, facts, and hypotheses. Life Sci. 1990;46(14):971-82.
140. Sugden D. Melatonin: binding site characteristics and biochemical and cellular responses. Neurochem Int. 1994;24(2):147-57.
141. Reppert SM, Weaver DR, Rivkees SA, Stopa EG. Putative melatonin receptors in a human biological clock. Science. 1988;242(4875):78-81.
142. Cagnacci A, Elliott JA, Yen SS. Melatonin: a major regulator of the circadian rhythm of core temperature in humans. J Clin Endocrinol Metab. 1992;75(2):447-52.
143. Cagnacci A. Influences of melatonin on human circadian rhythms. Chronobiol Int. 1997;14(2):205-20.
144. Dimenäs E, Kerr D, Macdonald I. Beta-adrenoceptor blockade and CNS-related subjective symptoms: a randomized, double-blind, placebo-controlled comparison of metoprolol CR/ZOK, atenolol and propranolol LA in healthy subjects. J Clin Pharmacol. 1990;30(2 Suppl):S103-7.
145. Harper DG, Stopa EG, McKee AC, Satlin A, Harlan PC, Goldstein R, et al. Differential circadian rhythm disturbances in men with Alzheimer disease and frontotemporal degeneration. Arch Gen Psychiatry. 2001;58(4):353-60.
146. Mishima K, Tozawa T, Satoh K, Matsumoto Y, Hishikawa Y, Okawa M. Melatonin secretion rhythm disorders in patients with senile dementia of Alzheimer's type with disturbed sleep-waking. Biol Psychiatry. 1999;45(4):417-21.

147. Kripke DF, Mullaney DJ, Atkinson M, Wolf S. Circadian rhythm disorders in manic-depressives. Biol Psychiatry. 1978;13(3):335-51.
148. Gale JE, Cox HI, Qian J, Block GD, Colwell CS, Matveyenko AV. Disruption of circadian rhythms accelerates development of diabetes through pancreatic beta-cell loss and dysfunction. J Biol Rhythms. 2011;26(5):423-33.
149. Coca A. Circadian rhythm and blood pressure control: physiological and pathophysiological factors. J Hypertens Suppl. 1994;12(5):S13-21.
150. Savvidis C, Koutsilieris M. Circadian rhythm disruption in cancer biology. Mol Med. 2012;18:1249-60. Epub 2012/12/06.
151. Khaper N, Bailey CDC, Ghugre NR, Reitz C, Awosanmi Z, Waines R, et al. Implications of disturbances in circadian rhythms for cardiovascular health: A new frontier in free radical biology. Free Radic Biol Med. 2017. Epub 2017/11/13.
152. Van Laake LW, Lüscher TF, Young ME. The circadian clock in cardiovascular regulation and disease: Lessons from the Nobel Prize in Physiology or Medicine 2017. Eur Heart J. 2017. Epub 2017/12/22.
153. Maywood ES, O'Neill J, Wong GK, Reddy AB, Hastings MH. Circadian timing in health and disease. Prog Brain Res. 2006;153:253-69.
154. Gradisar M, Wolfson AR, Harvey AG, Hale L, Rosenberg R, Czeisler CA. The sleep and technology use of Americans: findings from the National Sleep Foundation's 2011 Sleep in America poll. J Clin Sleep Med. 2013;9(12):1291-9. Epub 2013/12/15.
155. Gradisar MS, Michelle. Sleep Hygiene and Environment: Role of Technology. The Oxford Handbook of Infant, Child, and Adolescent Sleep and BehaviorDec 2013.
156. Roberts RE, Roberts CR, Duong HT. Sleepless in adolescence: prospective data on sleep deprivation, health and functioning. J Adolesc. 2009;32(5):1045-57. Epub 2009/04/09.
157. Aschoff J, Pohl H. Phase relations between a circadian rhythm and its zeitgeber within the range of entrainment. Naturwissenschaften. 1978;65(2):80-4.
158. Chang AM, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. Proc Natl Acad Sci U S A. 2015;112(4):1232-7. Epub 2014/12/22.
159. Cajochen C, Munch M, Kobialka S, Krauchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 2005;90(3):1311-6.
160. Wright HR, Lack LC, Partridge KJ. Light emitting diodes can be used to phase delay the melatonin rhythm. J Pineal Res. 2001;31(4):350-5.
161. Boivin DB, Duffy JF, Kronauer RE, Czeisler CA. Dose-response relationships for resetting of human circadian clock by light. Nature. 1996;379(6565):540-2.
162. Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res. 2000;115(1):75-83.
163. Wood B, Rea MS, Plitnick B, Figueiro MG. Light level and duration of exposure determine the impact of self-luminous tablets on melatonin suppression. Appl Ergon. 2013;44(2):237-40. Epub 2012/07/31.
164. Heath M, Sutherland C, Bartel K, Gradisar M, Williamson P, Lovato N, et al. Does one hour of bright or short-wavelength filtered tablet screenlight have a meaningful

effect on adolescents' pre-bedtime alertness, sleep, and daytime functioning? Chronobiol Int. 2014;31(4):496-505.
165. Burkhart K, Phelps JR. Amber lenses to block blue light and improve sleep: a randomized trial. Chronobiol Int. 2009;26(8):1602-12.
166. van der Lely S, Frey S, Garbazza C, Wirz-Justice A, Jenni OG, Steiner R, et al. Blue blocker glasses as a countermeasure for alerting effects of evening light-emitting diode screen exposure in male teenagers. J Adolesc Health. 2015;56(1):113-9.
167. Foster RG, Wulff K. The rhythm of rest and excess. Nat Rev Neurosci. 2005;6(5):407-14.
168. LeGates TA, Altimus CM, Wang H, Lee HK, Yang S, Zhao H, et al. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. Nature.491(7425):594-8. Epub 2012/11/16.
169. Warthen DM, Wiltgen BJ, Provencio I. Light enhances learned fear. Proc Natl Acad Sci U S A. 2011;108(33):13788-93. Epub 2011/08/01.
170. Vandewalle G, Collignon O, Hull JT, Daneault V, Albouy G, Lepore F, et al. Blue light stimulates cognitive brain activity in visually blind individuals. J Cogn Neurosci.25(12):2072-85. Epub 2013/07/19.
171. Viola AU, James LM, Schlangen LJ, Dijk DJ. Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. Scand J Work Environ Health. 2008;34(4):297-306. Epub 2008/09/22.
172. Roecklein KA, Rohan KJ, Duncan WC, Rollag MD, Rosenthal NE, Lipsky RH, et al. A missense variant (P10L) of the melanopsin (OPN4) gene in seasonal affective disorder. J Affect Disord. 2009;114(1-3):279-85. Epub 2008/09/23.
173. Iyilikci O, Aydin E, Canbeyli R. Blue but not red light stimulation in the dark has antidepressant effect in behavioral despair. Behav Brain Res. 2009;203(1):65-8. Epub 2009/04/18.
174. Meesters Y, Dekker V, Schlangen LJ, Bos EH, Ruiter MJ. Low-intensity blue-enriched white light (750 lux) and standard bright light (10,000 lux) are equally effective in treating SAD. A randomized controlled study. BMC Psychiatry. 2011;11:17. Epub 2011/01/28.
175. Glickman G, Byrne B, Pineda C, Hauck WW, Brainard GC. Light therapy for seasonal affective disorder with blue narrow-band light-emitting diodes (LEDs). Biol Psychiatry. 2006;59(6):502-7.
176. Noseda R, Kainz V, Jakubowski M, Gooley JJ, Saper CB, Digre K, et al. A neural mechanism for exacerbation of headache by light. Nat Neurosci. 2010;13(2):239-45. Epub 2010/01/10.
177. Johnson J, Wu V, Donovan M, Majumdar S, Renteria RC, Porco T, et al. Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A.107(40):17374-8. Epub 2010/09/22.
178. Matynia A, Parikh S, Chen B, Kim P, McNeill DS, Nusinowitz S, et al. Intrinsically photosensitive retinal ganglion cells are the primary but not exclusive circuit for light aversion. Exp Eye Res.105:60-9. Epub 2012/10/20.
179. Sikka G, Hussmann GP, Pandey D, Cao S, Hori D, Park JT, et al. Melanopsin mediates light-dependent relaxation in blood vessels. Proc Natl Acad Sci U S A.111(50):17977-82. Epub 2014/11/19.

180. Danilenko KV, Sergeeva OY. Immediate effect of blue-enhanced light on reproductive hormones in women. Neuro Endocrinol Lett. 2015;36(1):84-90.
181. Bergen MA, Park HN, Chakraborty R, Landis EG, Sidhu C, He L, et al. Altered Refractive Development in Mice With Reduced Levels of Retinal Dopamine. Invest Ophthalmol Vis Sci. 2016;57(10):4412-9.
182. Dkhissi-Benyahya O, Coutanson C, Knoblauch K, Lahouaoui H, Leviel V, Rey C, et al. The absence of melanopsin alters retinal clock function and dopamine regulation by light. Cell Mol Life Sci.70(18):3435-47. Epub 2013/04/23.
183. Cameron MA, Pozdeyev N, Vugler AA, Cooper H, Iuvone PM, Lucas RJ. Light regulation of retinal dopamine that is independent of melanopsin phototransduction. Eur J Neurosci. 2009;29(4):761-7. Epub 2009/02/10.
184. Jung SK, Lee JH, Kakizaki H, Jee D. Prevalence of myopia and its association with body stature and educational level in 19-year-old male conscripts in seoul, South Korea. Invest Ophthalmol Vis Sci. 2012;53(9):5579-83.
185. Rose KA, Morgan IG, Smith W, Mitchell P. High heritability of myopia does not preclude rapid changes in prevalence. Clin Experiment Ophthalmol. 2002;30(3):168-72.
186. Liang YB, Lin Z, Vasudevan B, Jhanji V, Young A, Gao TY, et al. Generational difference of refractive error in the baseline study of the Beijing Myopia Progression Study. Br J Ophthalmol. 2013;97(6):765-9.
187. Rose KA, Morgan IG, Ip J, Kifley A, Huynh S, Smith W, et al. Outdoor activity reduces the prevalence of myopia in children. Ophthalmology. 2008;115(8):1279-85. Epub 2008/02/26.
188. Read SA, Collins MJ, Vincent SJ. Light exposure and eye growth in childhood. Invest Ophthalmol Vis Sci. 2015;56(11):6779-87.
189. Ayaki M, Torii H, Tsubota K, Negishi K. Decreased sleep quality in high myopia children. Sci Rep. 2016;6:33902.
190. Jee D, Morgan IG, Kim EC. Inverse relationship between sleep duration and myopia. Acta Ophthalmol. 2016;94(3):e204-10.
191. Kearney S, O'Donoghue L, Pourshahidi LK, Cobice D, Saunders KJ. Myopes have significantly higher serum melatonin concentrations than non-myopes. Ophthalmic Physiol Opt. 2017;37(5):557-67.
192. Wong TY, Klein BE, Klein R, Knudtson M, Lee KE. Refractive errors, intraocular pressure, and glaucoma in a white population. Ophthalmology. 2003;110(1):211-7. Epub 2003/01/04.
193. Trejo LJ, Cicerone CM. Cells in the pretectal olivary nucleus are in the pathway for the direct light reflex of the pupil in the rat. Brain Res. 1984;300(1):49-62.
194. Roecklein KA, Wong PM, Miller MA, Donofry SD, Kamarck ML, Brainard GC. Melanopsin, photosensitive ganglion cells, and seasonal affective disorder. Neurosci Biobehav Rev. 2013;37(3):229-39.
195. Boatright JH, Hoel MJ, Iuvone PM. Stimulation of endogenous dopamine release and metabolism in amphibian retina by light- and K+-evoked depolarization. Brain Res. 1989;482(1):164-8.
196. McCarthy CS, Megaw P, Devadas M, Morgan IG. Dopaminergic agents affect the ability of brief periods of normal vision to prevent form-deprivation myopia. Exp Eye Res. 2007;84(1):100-7.

197. Gracitelli CP, Duque-Chica GL, Roizenblatt M, Moura AL, Nagy BV, Ragot de Melo G, et al. Intrinsically photosensitive retinal ganglion cell activity is associated with decreased sleep quality in patients with glaucoma. Ophthalmology. 2015;122(6):1139-48.
198. Maynard ML, Zele AJ, Kwan AS, Feigl B. Intrinsically Photosensitive Retinal Ganglion Cell Function, Sleep Efficiency and Depression in Advanced Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci. 2017;58(2):990-6.
199. Ismael ZF, El-Shazly AA, Farweez YA, Osman MM. Relationship between functional and structural retinal changes in myopic eyes. Clin Exp Optom. 2017.
200. Orr D, Mutti DO, Shorter P, Hartwick AT. The effect of light exposure on blue light-driven pupil responses in myopes and non-myopes. Optom Vis Sci. 2015;92:E-abstract 150035.
201. Meltzer LJ, Montgomery-Downs HE, Insana SP, Walsh CM. Use of actigraphy for assessment in pediatric sleep research. Sleep medicine reviews. 2012;16(5):463-75.
202. Ostrin LA. Objectively measured light exposure in emmetropic and myopic adults. Optom Vis Sci. 2017;94(2):229-38.
203. Ostrin LA, Abbott KS, Queener HM. Attenuation of short wavelengths alters sleep and the ipRGC pupil response. Ophthalmic Physiol Opt. 2017;in press.
204. Adhikari P, Zele AJ, Feigl B. The post-illumination pupil response (PIPR). Invest Ophthalmol Vis Sci. 2015;56(6):3838-49.
205. Mure LS, Rieux C, Hattar S, Cooper HM. Melanopsin-dependent nonvisual responses: evidence for photopigment bistability in vivo. J Biol Rhythms. 2007;22(5):411-24. Epub 2007/09/19.
206. Cajochen C, Krauchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. J Neuroendocrinol. 2003;15(4):432-7.
207. Karami Z, Golmohammadi R, Heidaripahlavian A, Poorolajal J, Heidarimoghadam R. Effect of Daylight on Melatonin and Subjective General Health Factors in Elderly People. Iran J Public Health. 2016;45(5):636-43.
208. Munch M, Ladaique M, Roemer S, Hashemi K, Kawasaki A. Melanopsin-Mediated Acute Light Responses Measured in Winter and in Summer: Seasonal Variations in Adults with and without Cataracts. Front Neurol. 2017;8:464.
209. Owen J, Arendt J. Melatonin suppression in human subjects by bright and dim light in antarctica: time and season-dependent effects. Neurosci Lett. 1992;137(2):181-4.
210. Heller PH, Perry F, Jewett DL, Levine JD. Autonomic components of the human pupillary light reflex. Invest Ophthalmol Vis Sci. 1990;31(1):156-62.
211. Li T, Troilo D, Glasser A, Howland HC. Constant light produces severe corneal flattening and hyperopia in chickens. Vision Res. 1995;35(9):1203-9.
212. Norton TT, Amedo AO, Siegwart JT, Jr. Darkness causes myopia in visually experienced tree shrews. Invest Ophthalmol Vis Sci. 2006;47(11):4700-7.
213. Smith EL, 3rd, Bradley DV, Fernandes A, Hung LF, Boothe RG. Continuous ambient lighting and eye growth in primates. Invest Ophthalmol Vis Sci. 2001;42(6):1146-52.
214. Li T, Howland HC, Troilo D. Diurnal illumination patterns affect the development of the chick eye. Vision Res. 2000;40(18):2387-93.
215. Ashby R, Ohlendorf A, Schaeffel F. The effect of ambient illuminance on the development of deprivation myopia in chicks. Invest Ophthalmol Vis Sci. 2009;50(11):5348-54.

216. Ashby RS, Schaeffel F. The effect of bright light on lens compensation in chicks. Invest Ophthalmol Vis Sci. 2010;51(10):5247-53.
217. Iuvone PM, Tosini G, Pozdeyev N, Haque R, Klein DC, Chaurasia SS. Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. Prog Retin Eye Res. 2005;24(4):433-56.
218. Read SA, Collins MJ, Vincent SJ. Light exposure and physical activity in myopic and emmetropic children. Optom Vis Sci. 2014.
219. Sakamoto K, Liu C, Kasamatsu M, Pozdeyev NV, Iuvone PM, Tosini G. Dopamine regulates melanopsin mRNA expression in intrinsically photosensitive retinal ganglion cells. Eur J Neurosci. 2005;22(12):3129-36. Epub 2005/12/22.
220. Schmid KL, Wildsoet CF. Inhibitory effects of apomorphine and atropine and their combination on myopia in chicks. Optom Vis Sci. 2004;81(2):137-47. Epub 2004/05/07.
221. Jiang L, Long K, Schaeffel F, Zhou X, Zheng Y, Ying H, et al. Effects of dopaminergic agents on progression of naturally occurring myopia in albino guinea pigs (Cavia porcellus). Invest Ophthalmol Vis Sci. 2014;55(11):7508-19.
222. Mutti DO, Marks AR. Blood levels of vitamin D in teens and young adults with myopia. Optom Vis Sci. 2011;88(3):377-82.
223. Loman J, Quinn GE, Kamoun L, Ying GS, Maguire MG, Hudesman D, et al. Darkness and near work: myopia and its progression in third-year law students. Ophthalmology. 2002;109(5):1032-8.
224. Nickla DL, Wildsoet C, Wallman J. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. Exp Eye Res. 1998;66(2):163-81. Epub 1998/06/17.
225. Moore RY. Organization and function of a central nervous system circadian oscillator: the suprachiasmatic hypothalamic nucleus. Fed Proc. 1983;42(11):2783-9.
226. Hardeland R, Pandi-Perumal SR, Cardinali DP. Melatonin. Int J Biochem Cell Biol. 2006;38(3):313-6.
227. Young RS, Kimura E. Pupillary correlates of light-evoked melanopsin activity in humans. Vision Res. 2008;48(7):862-71. Epub 2008/02/12.
228. Hartwick AT, Bramley JR, Yu J, Stevens KT, Allen CN, Baldridge WH, et al. Light-evoked calcium responses of isolated melanopsin-expressing retinal ganglion cells. J Neurosci. 2007;27(49):13468-80. Epub 2007/12/07.
229. Buijs RM, Chun SJ, Niijima A, Romijn HJ, Nagai K. Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. J Comp Neurol. 2001;431(4):405-23.
230. Kalsbeek A, Fliers E, Franke AN, Wortel J, Buijs RM. Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. Endocrinology. 2000;141(10):3832-41.
231. Ben-Shlomo R. Chronodisruption, cell cycle checkpoints and DNA repair. Indian J Exp Biol. 2014;52(5):399-403.
232. Reid KJ, Zee PC. Circadian rhythm sleep disorders. Handb Clin Neurol. 2011;99:963-77.
233. Monteleone P, Martiadis V, Maj M. Circadian rhythms and treatment implications in depression. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(7):1569-74.

234. Schwartz JR, Roth T. Shift work sleep disorder: burden of illness and approaches to management. Drugs. 2006;66(18):2357-70.
235. Erren TC, Gross JV, Meyer-Rochow VB. Light, clocks, mood, and cancer: consolidation and novel tests of latitude and instability hypotheses. Chronobiol Int. 2011;28(5):471-3; author reply p 3.
236. Van Someren EJ, Riemersma-Van Der Lek RF. Live to the rhythm, slave to the rhythm. Sleep medicine reviews. 2007;11(6):465-84.
237. Brainard GC, Hanifin JP, Warfield B, Stone MK, James ME, Ayers M, et al. Short-wavelength enrichment of polychromatic light enhances human melatonin suppression potency. J Pineal Res. 2015;58(3):352-61.
238. Mai E, Buysse DJ. Insomnia: prevalence, impact, pathogenesis, differential diagnosis, and evaluation. Sleep medicine clinics. 2008;3(2):167-74.
239. Mottram AR, Svenson JE. Rhythm disturbances. Emerg Med Clin North Am. 2011;29(4):729-46, vi.
240. Cajochen C, Dijk DJ, Borbely AA. Dynamics of EEG slow-wave activity and core body temperature in human sleep after exposure to bright light. Sleep. 1992;15(4):337-43.
241. Cajochen C, Frey S, Anders D, Spati J, Bues M, Pross A, et al. Evening exposure to a light-emitting diodes (LED)-backlit computer screen affects circadian physiology and cognitive performance. Journal of applied physiology. 2011;110(5):1432-8.
242. Kayumov L, Casper RF, Hawa RJ, Perelman B, Chung SA, Sokalsky S, et al. Blocking low-wavelength light prevents nocturnal melatonin suppression with no adverse effect on performance during simulated shift work. J Clin Endocrinol Metab. 2005;90(5):2755-61.
243. Voultsios A, Kennaway DJ, Dawson D. Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms. 1997;12(5):457-66.
244. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989;28(2):193-213.
245. Barrionuevo PA, Nicandro N, McAnany JJ, Zele AJ, Gamlin P, Cao D. Assessing rod, cone, and melanopsin contributions to human pupil flicker responses. Invest Ophthalmol Vis Sci.55(2):719-27. Epub 2014/01/11.
246. Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, et al. Measuring and using light in the melanopsin age. Trends Neurosci. 2014;37(1):1-9.
247. Perlman I, Normann RA. Light adaptation and sensitivity controlling mechanisms in vertebrate photoreceptors. Prog Retin Eye Res. 1998;17(4):523-63.
248. Zhu Y, Tu DC, Denner D, Shane T, Fitzgerald CM, Van Gelder RN. Melanopsin-dependent persistence and photopotentiation of murine pupillary light responses. Invest Ophthalmol Vis Sci. 2007;48(3):1268-75. Epub 2007/02/28.
249. Adhikari P, Pearson CA, Anderson AM, Zele AJ, Feigl B. Effect of Age and Refractive Error on the Melanopsin Mediated Post-Illumination Pupil Response (PIPR). Sci Rep.5:17610. Epub 2015/12/02.
250. Herbst K, Sander B, Lund-Andersen H, Broendsted AE, Kessel L, Hansen MS, et al. Intrinsically photosensitive retinal ganglion cell function in relation to age: a

pupillometric study in humans with special reference to the age-related optic properties of the lens. BMC Ophthalmol.12:4. Epub 2012/04/05.
251. Dharani R, Lee CF, Theng ZX, Drury VB, Ngo C, Sandar M, et al. Comparison of measurements of time outdoors and light levels as risk factors for myopia in young Singapore children. Eye (Lond). 2012;26(7):911-8.
252. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. A phase response curve to single bright light pulses in human subjects. J Physiol. 2003;549(Pt 3):945-52.
253. Esaki Y, Kitajima T, Ito Y, Koike S, Nakao Y, Tsuchiya A, et al. Wearing blue light-blocking glasses in the evening advances circadian rhythms in the patients with delayed sleep phase disorder: An open-label trial. Chronobiol Int. 2016;33(8):1037-44.
254. Brondsted AE, Haargaard B, Sander B, Lund-Andersen H, Jennum P, Kessel L. The effect of blue-blocking and neutral intraocular lenses on circadian photoentrainment and sleep one year after cataract surgery. Acta Ophthalmol. 2016.
255. Brondsted AE, Sander B, Haargaard B, Lund-Andersen H, Jennum P, Gammeltoft S, et al. The effect of cataract surgery on circadian photoentrainment: a randomized trial of blue-blocking versus neutral intraocular lenses. Ophthalmology. 2015;122(10):2115-24.
256. Lichstein KL, Stone KC, Donaldson J, Nau SD, Soeffing JP, Murray D, et al. Actigraphy validation with insomnia. Sleep. 2006;29(2):232-9.
257. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. Sleep. 2003;26(3):342-92.
258. Rahman SA, Flynn-Evans EE, Aeschbach D, Brainard GC, Czeisler CA, Lockley SW. Diurnal spectral sensitivity of the acute alerting effects of light. Sleep. 2014;37(2):271-81.
259. West KE, Jablonski MR, Warfield B, Cecil KS, James M, Ayers MA, et al. Blue light from light-emitting diodes elicits a dose-dependent suppression of melatonin in humans. Journal of applied physiology. 2011;110(3):619-26.
260. Mantua J, Gravel N, Spencer RM. Reliability of sleep measures from four personal health monitoring devices compared to research-based actigraphy and polysomnography. Sensors. 2016;16(5).
261. Yamasaki A, Kawasaki Y, Takeda K, Harada T, Fukushima T, Takata M, et al. The relationships among sleep efficiency, pulmonary functions, and quality of life in patients with asthma. Int J Gen Med. 2014;7:505-12.
262. Kuo HH, Chiu MJ, Liao WC, Hwang SL. Quality of sleep and related factors during chemotherapy in patients with stage I/II breast cancer. J Formos Med Assoc. 2006;105(1):64-9.
263. Okudaira N, Kripke DF, Webster JB. Naturalistic studies of human light exposure. Am J Physiol. 1983;245(4):R613-5.
264. Jasser SA, Hanifin JP, Rollag MD, Brainard GC. Dim light adaptation attenuates acute melatonin suppression in humans. J Biol Rhythms. 2006;21(5):394-404.
265. Ferracioli-Oda E, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One. 2013;8(5):e63773. Epub 2013/05/17.