Understanding Circadian Responses to Light in Persons With Mild Cognitive Impairment and Alzheimer's Disease
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SPECIFIC AIMS (*resubmission revisions marked in italics*)

In 2019, the estimated total cost associated with ADRD in the United States was $234 billion, which is expected to balloon to $1.1 trillion by 2050, according to the latest (2019) estimates by the Alzheimer's Association. [2] Approximately 40% of the estimated 5.5 million older (65+) Americans with Alzheimer's disease and related dementias (ADRD) suffer from severe dysfunction of their sleep-wake and circadian systems, manifesting clinically as sundowning, excessive daytime sleepiness, nocturnal wandering, agitation, and day-night reversal. [3,4] In people with mild cognitive impairment (MCI), an "at risk" or potential prodromal stage of dementia, sleep-wake disturbance is evident in up to 60% of patients. [5,6]

The daily pattern of light and dark reaching our retinas is the primary *zeitgeber* that regulates distinct biological processes by setting the timing of the endogenous circadian pacemaker in the suprachiasmatic nucleus (SCN) in accordance with Earth's 24-h solar day. Melatonin is produced by the pineal gland at night in dark conditions and is known as the "darkness hormone." In humans, melatonin is used as a marker of the timing of the biological clock and its production is ceased by exposure to light at night, which is one of the main reasons melatonin is used as an outcome measure in experiments designed to understand circadian phototransduction mechanisms in humans.[7,8] Circulating melatonin levels change in a graded manner to variations in spectrum, amount, distribution, duration, and timing of light exposure. It is therefore imperative that a complete understanding of these lighting characteristics be developed so that: (1) the most effective lighting is used for therapy and (2) the most appropriate and effective light is installed in buildings. The latter application is particularly significant because senior housing lighting is typically dim and constant, which could be a reason why sleep disturbances are so much more common in older adults, including those with Alzheimer's disease and related dementias (ADRD) who are living in more controlled environments. Much is already known about the absolute and spectral sensitivities[9,10,11] of acute melatonin suppression, but not much attention has been given to the effect of light's spatial characteristics (distribution) and phenotype (macular pigment optical density or MPOD). The present application will characterize how light distribution on the retina and MPOD affect melatonin suppression. **We hypothesize that light incipient on the retina in central vision (i.e., at the fovea) will be more effective at suppressing melatonin than light reaching the periphery of the retina, and that melatonin suppression will be inversely correlated with MPOD**. We further hypothesize that, compared to age-matched controls, mild cognitive impairment (MCI) patients will have lower MPOD than age-matched controls. Finally, we will explore the relationship between MPOD and cognition and the feasibility of using MPOD as a biomarker of individual light sensitivity. The following aims will be investigated:

Specific aim 1: To determine how retinal light distribution impacts melatonin suppression in patients with MCI and in age-matched controls: We will investigate whether changing light distribution pattern from "on-axis"' (i.e., directed along the eye's visual axis to the fovea) to "off-axis" (i.e., directed on the periphery of the eye's visual axis) impact melatonin suppression. Given the retinal morphology and the highest density of photoreceptors in the 2° field of view, we hypothesize that, for the same light level, light delivered on-axis will be more effective at suppressing melatonin than light delivered off-axis.

Specific aim 2: To investigate how macular pigment optical density (MPOD) affects melatonin suppression in patients with MCI and in age-matched controls: The macula, an oval-shaped, yellow pigmented area located at the center of the retina, preferentially filters out short-wavelength optical radiation with a peak absorbance around the same wavelength as the peak spectral sensitivity of the human circadian system (460 nm). [1] We hypothesize that melatonin suppression will be inversely correlated with MPOD measurements.

Specific aim 3: To investigate how MPOD is associated with cognition. *Given that MPOD has been shown to be related to cognition (based on mini-mental state examination composite scores, visual-spatial and constructional abilities, language ability, attention, and the total scale on the Repeatable Battery for the Assessment of Neuropsychological Status), [12] we also hypothesize that MCI patients will have lower MPOD scores and that MPOD measurements will be negatively associated with cognition, as measured by the Montreal Cognitive Assessment (MoCA), Digital symbol substitution, Digital Span and Verbal Fluency.*

Findings from the proposed research project will provide feasibility and efficacy for future studies designed to test a more comprehensively tailored lighting intervention to promote circadian entrainment and improve sleep consolidation in persons with ADRD, who typically spend a significant amount of time indoors, with

little daytime light exposure and perhaps too much nighttime light exposure. Currently, the tailored lighting intervention used in our previous studies only takes into account light's spectrum and amount and is not optimized for light distribution in the retina. A secondary outcome will be a better understanding of how MPOD may be associated with cognitive decline. **These results will help us develop light-based interventions designed to deliver a robust light-dark pattern to MCI and ADRD patients, which will promote better sleep and better cognition**. The proposed exploratory study is directly responsive to the goals of **PA-18-347**.

RESEARCH STRATEGY

Background

Approximately 40% of the estimated 5.5 million older (65+) Americans with Alzheimer's disease and related dementias (ADRD) suffer from severe dysfunction of their sleep-wake and circadian systems, manifesting clinically as sundowning, excessive daytime sleepiness, nocturnal wandering, agitation, and day-night reversal. [3,4] *Recent animal and human studies suggest a bi-directional relationship wherein circadian and sleep disruption can also influence AD onset and progression. An increase in amyloid plaque deposition has been reported due to chronic sleep deprivation, with sleep extension protocols reporting lower plaques.* [13,14] In people with mild cognitive impairment (MCI), an "at risk" or potential prodromal stage of dementia, sleep-wake disturbance is evident in up to 60% of patients. [5,6] Sleep is critical for healthy cognitive processing, including working and long-term memory, with specific neural events recorded from scalp electroencephalography (EEG) associated with memory enhancement. Poor sleep and decreased memory-related sleep EEG events have been associated with decreased memory functioning in both groups. [15] Importantly, light treatment has been shown to improve sleep in older adults, and our group has preliminary evidence from a light treatment study in AD patients of improved sleep, mood, and behavior. *The specification of lighting for these interventions has mainly been based on light's amount and spectrum, and has not taken retinal light distribution into account. One of the issues with this approach is the risk of glare and discomfort. A better understanding of the retinal sensitivity would allow for the development of devices delivering more precise, and therefore, more effective doses.*

Sleep, circadian rhythms and light. The sleep/wake pattern is directly driven by the timing signals generated by the suprachiasmatic nuclei (SCN), which is known to be compromised by aging and ADRD. Studies have shown a reduced circadian rhythm amplitude after the age of 50.[16,17] It is hypothesized that some of the neural processes involved in entrainment might become dysfunctional or less effective as we age.[18] Disturbances in circadian rhythms leading to poor sleep in older adults can be the result of dysfunctional circadian pathways or a pathway that cannot process light information with as much fidelity. Also, the first stage of phototransduction (when light signals are converted into neural signals) is negatively affected. Older adults not only have reduced optical transmission at short wavelengths, which is maximally effective for the circadian system, they also lead a more sedentary indoor lifestyle, with less access to bright light during the day. In fact, research demonstrates that middle-aged adults receive approximately 58 minutes of bright light per day[19] while older adults in assisted-living facilities receive bright light for only 35 minutes per day.[20] Finally, changes in the amplitude and timing of melatonin and core body temperature rhythms may occur in older adults. Lower amplitude of melatonin rhythms may be associated with reduced sleep efficiency and deterioration of internal circadian rhythms, such as hormone production, alertness, and performance.[21,22,23] In order to maintain synchronization in the face of these physiological changes, it is necessary both to increase the strength of the light stimulus and to design an intervention that is maximally effective for entraining the circadian systems of those with MCI and ADRD. A 24-h light-dark pattern incident on the retina is the most efficacious stimulus for entraining the circadian system in humans.[24] Indeed, a carefully orchestrated light-dark pattern has been shown to be a powerful nonpharmacological tool to improve sleep efficiency and consolidation in several controlled studies of older populations, with and without ADRD.[25-27] More recently, work from our own laboratory showed increased sleep efficiency and reduced depression and agitation behavior after only 4 weeks of a tailored lighting intervention.[28] *However, current approaches to light therapy for reducing sleep disturbances as well as depressive and agitation symptoms in MCI and ADRD patients do not consider the non-uniform photoreceptors' mosaic in the retina, and light therapy tends to be too bright and uncomfortable for users. This proposal will lay the foundation for the development of more precise light treatment devices to effectively promote better sleep and quality of life in this population.*

Non-uniform photoreceptors' mosaic across retina: The retina is a complex neural network lining the back of the eye, made up of numerous nerve cell bodies segmented into 3 distinct layers: ganglion cell layer (GCL)

where the intrinsically photosensitive retina ganglion cells (ipRGCs) are located, the inner nuclear layer (INL) composed of the collector cells, and the outer nuclear layer (ONL) where the classical photoreceptors (rods and cones) are located. Our visual acuity (e.g., ability to read black text on white paper) is characterized by long (L) and middle (M) cone responses. The human circadian system, however, has a spectral sensitivity close to 460 nm, which falls within the sensitivity range of the short (S) cones. Even though the ipRGCs are the principal conduit of neural signals from the retina to the SCN, [29,30] they also receive and integrate signals from the classical photoreceptors. [31,32] More importantly, each of these photoreceptor classes involved in circadian phototransduction has a distinct distribution as a function of retinal eccentricity, which creates a non-uniform photoreceptors mosaic within the retina. Cumulatively, for most of the retina, rod photoreceptor density is substantially higher compared to the cone photoreceptor density. [33-35] However, an inverse trend can be observed in the fovea, wherein the cone density increases almost 200-fold with a concomitant sharp decline in rod density. The distribution across the retina is also quite different for the 3 cone types. The L- and M-cones are densely packed in the fovea, with a sharp decline in their density with increasing retinal eccentricity. [33-35] The S-cone density, although reported to be low in the fovea, peaks in the parafoveal region, followed by a gradual decline with a subsequent increase in retinal eccentricity. *Thus, in theory, it is possible that different portions of the retina illuminated by same photic stimulus will yield circadian responses of varying magnitudes.*

Past studies looking at lighting distribution and circadian system's response: Despite well-established research on the topography of photoreceptors across the retina, there is a troubling dearth of research on the spatial sensitivity of the human circadian system. No studies over the past 15 years have consistently and conclusively determined how lighting distribution at the eye, and more importantly, on the retina, modulates the circadian efficacy of the incident dose. For example, an early study by Adler, et al. [36] treated 12 healthy subjects to bright light exposure (1000 lux) under 2 spatial lighting scenarios: central (5°) and laterally peripheral (60°), but failed to report a significant difference in melatonin suppression between the 2 lighting conditions. An another study concluded that illuminating the entire retina is more effective for suppressing melatonin than illuminating (at equal photon dose) only the retina's inferior portion. [37] Some studies found that illuminating the inferior portion of the retina was more effective for suppressing melatonin than the superior portion,[12] a finding that was not supported by a subsequent study.[38] Taking a different approach, another study involving subjects exposed to either a monocular or binocular light stimulus, found that exposure to a white light source (630 lx) induced significantly greater melatonin suppression when both eyes were stimulated compared to just one eye. [39,40] Despite these disparate findings, and the somewhat rudimentary methods for estimating the retinal exposures used by the studies to date, it is nonetheless generally understood that *the human circadian system's ability to weigh and integrate photic stimuli spatially, and subsequent regulation of melatonin synthesis, is bound to be affected by the physical orientation of a light source and the gaze behavior relative to the light source.*

Macular pigment optical density (MPOD): In addition to the photoreceptors, the density of the short-wavelength optical radiation attenuating macular pigment has also been shown to vary across the retina. Typically, the concentration of the macular pigment is highest at, or near, the fovea and rapidly decreases with an increase in eccentricity. In healthy adults, MPOD has been shown to vary by almost one log unit (0.1–1.0), and that the within population MPOD variance can be largely explained by factors such as diet, age, and genetics[41]. *Therefore, MPOD may be used as a biomarker for individual sensitivity to light treatment.*

Relationship between macular carotenoids and cognitive function: The macular carotenoids lutein (L) and zeaxanthin (Z) preferentially accumulate in the central retina where their concentrations can be reliably assessed by measuring MPOD. [42] L and Z carotenoids have been shown to breech the blood–brain barrier and accumulate in various diffuse brain regions, with an especially high correlation reported between retinal L and Z levels and those estimated in occipital brain areas. [43-46] In other words, a close relationship between the macular carotenoids (and the retina itself) and the rest of the central nervous system [47-50] has enabled the researchers to employ MPOD as a proxy for neural L and Z concentrations. [45] More importantly, a number of studies have shown that higher concentrations of L and Z, as measured by higher MPOD values, have been significantly associated with improved cognitive performance, [47,51-57] potentially by strengthening neural structure and augmenting neural efficiency. [49] For example, a recent study has reported significantly lower performance on global measures of cognitive functioning (based on Mini-Mental Status Exam and Montreal Cognitive Assessment outcomes) in older adults with low MPOD values. [58] MPOD has also been associated with other cognitive functions regulating attention, visual memory, learning, and cognitive flexibility measuring subject's

ability to switch tasks. [54] Taking a different approach, an exploratory clinical trial found that supplementing older females with L improved their verbal fluency and performance on memory tests. [59] More recently, a placebo-controlled trial involving older adults found that L and Z dietary supplements improved performance on measures of complex attention and cognitive flexibility. [52] Overall, an increase in MPOD by even about 0.09 log units through dietary supplementation has been shown to improve cognition. [57] *In this feasibility study, we will investigate the exploratory hypothesis that MCI patients who have lower MPODs and lower cognition will be more sensitive to light at night and suppress more melatonin when light is delivered on-axis, which will lead to circadian disruption.*

Significance and Innovation

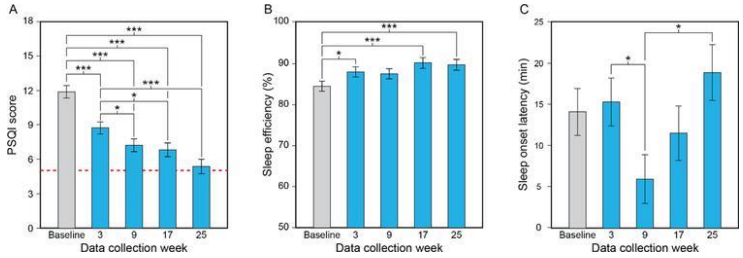

*Fig. 1. Tailored lighting intervention (TLI) during a 6-month randomized single-arm design clinical trial involving 47 persons with ADRD living in long-term care facilities improved measures of sleep. The error bars represent standard error of the mean. *** p<0.001, ** p<0.01 and * p<0.05*

The proposed study will 'shed light' on a relatively unexplored avenue of circadian phototransduction and spatial sensitivity, an understanding of which is essential for specifying and implementing a lighting design in healthcare facilities, senior care living facilities, and residences of MCI and ADRD patients. Findings from the proposed research project will be used to expand upon the Lighting Research Center 's previous related work formulating an extensive mathematical model of human circadian phototransduction ("CS model") [60] based on the effects of retinal light exposures on nocturnal melatonin suppression, which is consistent with the neuroanatomy and physiology of the human retina. The model has been extensively used in the laboratory and the field to predict efficacy of light exposures on clinically relevant outcome measures, such as sleep onset time, demonstrating its scientific and face validity. The model does not, however, take into account human retinal sensitivity resulting from non-uniform photoreceptor distribution. **The results of the proposed research would inform the development of light-delivery methods to promote circadian entrainment and improve sleep consolidation in patients afflicted with MCI and ADRD, who typically spend a significant amount of time indoors.** Several studies in the literature have associated AD with an increase in circadian disruption. [61,62] Prior work by Dr. Figueiro (PI) has shown that appropriately timed light exposure can increase sleep efficiency (**Fig**. 1), decrease agitation, and even limit cognitive decline in the ADRD population, [63] but the light delivery method and strategy has been identified as one of the greatest challenges for successful treatment. Results from the proposed research are also important to this specific application, as past studies have reported significantly lower MPODs in MCI and ADRD populations. [64] Finally, we will be able to better understand whether MPOD affects cognition and explore the possibility that MPOD could be used as a biomarker for individual sensitivity to light, allowing for the development of individualized light treatment prescriptions to more effectively entrain the circadian system, thus, improving sleep and cognition.

Following advancements in LED technology that allow more dynamic distribution patterns for light sources, the proposed work marks an important shift in approach moving beyond the conventional "light at the cornea" for circadian lighting to "light at the retina." The meticulously planned study will test a novel strategy of whether modulating lighting distribution at the eye provides a more efficient way to manipulate circadian stimulus delivered, compared to amount or spectral modifications for light sources, which often are governed by requirements to satisfactorily perform visual tasks. The state-of-the-art custom-built lighting apparatus will allow us to selectively involve and study interaction of MPOD with the varying lighting characteristics (spectrum, distribution), which is unique as no prior studies have examined the correlation between MPOD and melatonin suppression. Lastly, the target spectra for the narrowband sources have been selected so that photic stimulus incident on the retina will be attenuated by the macular pigment (peak absorbance = 460 nm) only for the blue light and not the green light exposures proposed for the study (see Experimental Conditions).

Approach

Study Team: Study Team: Mariana Figueiro, PhD, Professor of Architecture and Biological Sciences at RPI and Director of the Lighting Research Center, specializes in human circadian responses to light. She is currently a PI on an R01 investigating the impact of light on sleep efficiency of older adults with ADRD living in nursing homes. She is also a PI on another R01 investigating the impact of a tailored lighting intervention

technology on metabolic syndrome in ADRD and MCI patients. **Rohan Nagare, PhD,** is a research scientist with expertise in circadian phototransduction mechanisms and modeling. **Mark Rea, PhD,** Professor of Cognitive Sciences and Architecture at RPI, has been actively involved in modeling human visual response to light, and his accomplishments include the development of the first model of human circadian phototransduction. **Barbara Plitnick, RN,** is the Manager of Light and Health Program who has over 15 years of experience recruiting and running studies for a series of light and health projects.

Preliminary Data:

Role of MPOD in circadian photoreception: Nagare et al. have recently conducted a series of within-subjects nighttime studies recording light-induced melatonin suppression in human subjects exposed for 1 h to white light sources with CCTs ranging from 3300 K to 5000 K. Measurements for MPOD were performed using a non-invasive instrument employing psychophysical optical technique based on heterochromatic flicker photometry to determine MPOD's role in circadian photoreception. It was observed that a higher MPOD was significantly correlated with lower melatonin suppression, wherein the macular pigment acted as a filter, lowering the absolute circadian sensitivity **(Fig. 2). The effect size of this observation further indicated that about 35% of the light incident on cornea was being modulated by the macular pigment for the rectangular light sources used in the study**.

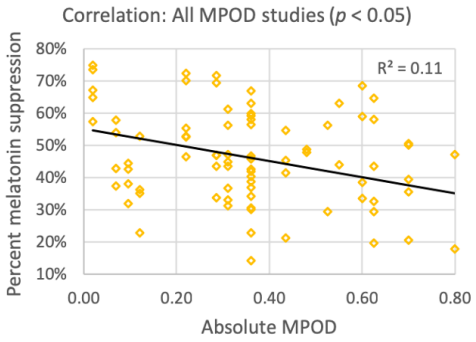

Fig. 2. Correlation between MPOD and melatonin suppression showing pooled data from a series of within-subjects nighttime studies. Each yellow diamond corresponds to suppression data for an individual subject. The black line corresponds to the best fit linear regression model. All subjects underwent a 1-h exposure to white light sources with CCTs ranging

These studies were performed using healthy young adults and our proposal will extend these results to include older adults, including those with MCI, who have been shown to be less sensitive to light overall. Moreover, preliminary data from our lab using a protocol similar to the one described here (results described in introduction) showed that light delivered on axis is almost twice as effective as light delivered off axis. Therefore, in order to deliver a robust light-dark pattern to promote entrainment and minimize nighttime melatonin suppression, a better understanding of the retinal mechanisms associated with circadian phototransduction is needed.

Participant selection: Following IRB approval, we will recruit 24 MCI participants and 24 healthy, age-matched controls through e-mail notices, posters, and word-of-mouth. MPOD will be assessed at the recruitment stage for all subjects to determine their initial eligibility, which will require subjects to fit into one of 2 distinct groups (low: MPOD < 0.3, high: MPOD > 0.5). Eligible participants will be MCI or mild AD patients who are 55 years and older. We will work with our physician collaborators in the area (who are currently working with the PIs in 3 other R01s) to perform the pre-selection diagnosis according to the following **pre-selection criteria**: Participants must be diagnosed by their physician with amnestic MCI or mild AD. Subjects in the control group will have normal cognition. Study participants who meet the following inclusion and exclusion will discuss informed consent for study participation: ***Inclusion criteria:*** Those taking antidepressants will be included, but type of medicine and dosage intake will be monitored. There will be no exclusions based gender, race, and ethnicity. Participants must also not meet the following ***Exclusion criteria:*** Presence of another brain disease that fully explains the dementia (extensive brain vascular disease, Parkinson's disease, dementia with Lewy bodies, traumatic brain injury, or multiple sclerosis); residence in a skilled nursing facility or long-term care; indication for psychiatric hospitalization or acute suicidality in the opinion of the physician; major organ failure (e.g., kidney failure); or uncontrolled generalized disorders such as hypertension or diabetes. Exclusion criteria will also extend to obstructing cataracts, macular degeneration, and blindness. Those taking over-the-counter melatonin, prescription sleep medication or beta blockers will be excluded. We will review the participants' ophthalmologic tests during selection and exclude those who have undergone

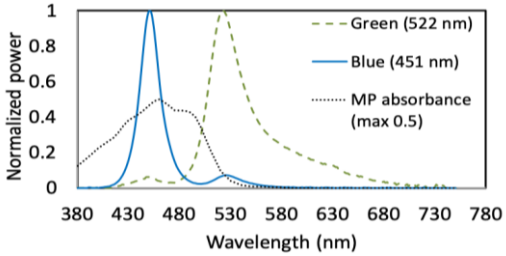

Fig. 3. Relative spectral power distributions of potential narrowband blue light source (λ_{max} = 451 nm) and green light source (λ_{max} = 522 nm). Also shown is the relative spectral absorbance of the macular pigment, adapted

cataract surgery and received an intraocular lens coated with ultraviolet- and blue-blocking filters (400-440/440-500 nm). Those with a history of severe photosensitivity dermatitis, severe progressive retinal disease (e.g., macular degeneration), or a permanently dilated pupil (e.g., after certain types of cataract surgery) will also be excluded. Subjects will also be required to maintain a regular sleep schedule (bedtimes no later than 23:00 and wake times no later than 08:00) starting 2 weeks prior to the experimental sessions and continuing throughout the experiment's duration. The experimenters will run the dim-light control condition first, and any subjects who present low nighttime (i.e., at 24:00) melatonin levels will be excluded from the study. This study will conform to the Code of Federal Regulations (CFR) document Protection of Human Subjects, 45 CFR 46, [67] and International Ethical Standards. [68]

Experimental conditions: In addition to a dim-light control condition, participants will be exposed to 2 narrowband sources: blue light (λ_{max} = 451 nm) and green light (λ_{max} = 522 nm) (**Fig. 3**), presented in 4 interventions. The 2 interventions for each narrowband source, designated on-axis and off-axis, will have distinct lighting distribution patterns but calibrated to deliver the same targeted levels of circadian light (CL_A) and circadian stimulus (CS) at the eye. [60,69] The 4 interventions will target a CS = 0.40 (≈ 27 lux for blue light, ≈ 250 lux for green light) at the participants' eyes and the dim light control will target a CS < 0.1 (< 5 lux of 2700 K light) at the eyes. The 4 lighting conditions will be counter-balanced across all the participants. Over the course of the study, spectral irradiance measurements for the 2 narrowband sources will be performed using a spectrometer (Model USB650, Ocean Optics, Winter Park, FL, USA).

Lighting apparatus: The on-axis and off-axis lighting interventions will be delivered via RGB color-tunable LED luminaires (model G2, Ketra, Austin, TX), each driven by a satellite link controller (model N3, Ketra) with a touchpad interface (model X1, Ketra) and housed in fixtures that will be placed directly on desks at which the participants will be seated. The luminaires will be covered by spectrally neutral diffusers to eliminate potential glare. The intervention stimulus will be calibrated using a tripod-mounted illuminance meter (Model X-91, Gigahertz-Optik, Haverhill Rd, Amesbury, MA, USA). The target photometric characteristics of the intervention light stimulus for measures of illuminance, CL_A, and CS following Rea and Figueiro [70], and equivalent melanopic lux (EML) following Enezi, et al. [71] are shown in Table 1. To ensure consistency of the delivered lighting dose, participants will be required to use a chin rest, optimally adjusted to maintain a fixed distance of 30 in. from the 22 in. x 5 in. (w × h) light-emitting area of the desktop luminaire (**Fig. 4**). The on-axis intervention will provide an image ± 5° in width at each participant's eyes. The off-axis intervention will provide an image ± 8–20° in width at each participant's eyes.

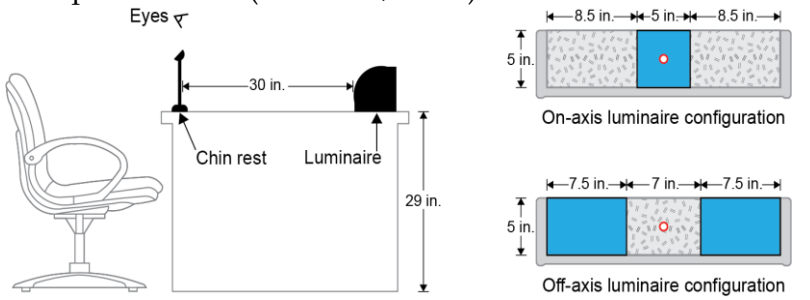

Fig. 4. The layout of the desktop luminaires with respect to the participants' eyes during the experiment (left), and the luminaires' configuration when delivering the on-axis and off-axis interventions (right). The circle shown in the center of the luminaires represents a low-power LED that will be used as a simple on-axis visual task to maintain participants line of sight.

Table 1. Photometric characteristics of the target blue light and green light stimulus calculated following Rea and Figueiro [70] (CS) and Enezi, et al. [71] (EML)

Experimental condition	Photopic illuminance (lux)	CL_A	EML	Predicted CS
Blue on-axis /Blue off-axis	26.7	473.7	154.0	0.40
Green on-axis /Green off-axis	250.0	473.7	274.0	0.40

Participant measurements and field monitoring procedures:

Macular pigment optical density (MPOD): Measurements for MPOD will be performed using a non-invasive instrument (*QuantifEye MPS II, ZeaVision (LLC), Chesterfield, MO*) employing well-established psychophysical optical technique based on heterochromatic flicker photometry. [72] ***Pupil measurement****: Participants' pupil diameters will be estimated while they are exposed to all interventions using a digital video camcorder (model DCR-TRV140, SONY Electronics, Minato, Tokyo, Japan) and subsequent frame-wise analyses using an open-source web application (Pixel*

Ruler, MIOPlanet Technologies, Rimouski, PQ, Canada) that estimates pupil diameter for 5 samples per 15-s video segment. We will then calculate the retinal illuminance. The simplified formula is T = LS, where L is the luminance of the stimulus cd/m² and S is the area of the pupil in mm². The retinal illuminance T is given in trolands. ***Sleep/wake diary****:* Subjects will maintain a sleep/wake diary during the entire study. These diaries will document bedtimes, rising times, and caffeine intake. ***Actigraphy****:* The actigraph is a device the size of a digital wrist watch (1.75" x 1.3" x 0.38", weighing 2 oz) that is worn on the nondominant wrist (Actiwatch, Philips Respironics, Murrysville, PA, or similar). Subjects will begin to wear the device 7 days prior to starting the protocol and throughout the duration of the data collection period. When subjects return to the laboratory for the experimental sessions, the actigraph data will be downloaded and compared to the diary information. Discrepancies will be identified and the actigraph record will be annotated. The data will be analyzed to estimate sleep/wake using the manufacturer's software. ***Cognitive tests:*** *In addition to MOCA (described above), participants will be asked to perform the following tests at recruitment:* ***Digit Symbol Substitution (DSST):*** *The DSST requires response speed, sustained attention, and set shifting (executive function). The participant is given 90 seconds to pair correctly numbers with symbols; the higher the score the better the performance.* ***Digit Span:*** *Measures working memory's number storage capacity. The participant hears a sequence of numerical digits and is tasked to recall the sequence correctly. Digit span is the longest number of sequential digits that can be accurately remembered.* ***Verbal Fluency:*** *Participants have to produce as many words as possible starting with a given letter under various rule constraints. Successful retrieval requires executive control over cognitive processes such as selective attention and inhibition, mental set shifting, internal response generation and self-monitoring.*

Melatonin Suppression Protocol: On each study night, participants will be required to arrive at the laboratory at 23:30 and remain in dim light (< 5 lux at the eye) for 30 min, followed by a 60-min exposure to one of the 4 experimental conditions (**Fig. 5**). To ensure consistency of the light distribution with regard to the participants' line of sight over the course of the 1-h exposure, all participants will perform a simple on-axis visual task that involves responding (using a hand-held tally counter) to a flashing, pre-programmed RGB LED source (generic low-power) suspended in the center of the desktop luminaire (see Fig. 4). The visual task will not be performed on the dim-light control night, when participants will be free to operate their personal electronic devices (i.e., iPads, tablets, cell phones, etc.). However, all displays will be covered with orange-tinted media (Roscolux #21 golden amber, Rosco Laboratories, Stamford, CT, USA) to filter out radiation < 525 nm to prevent participants from receiving circadian-effective light from their self-luminous devices. In a previous study, photometric measurements of the stimulus emitted by similarly filtered electronic displays revealed light levels of < 5 lux, which translated to a CS of < 0.001 [73].

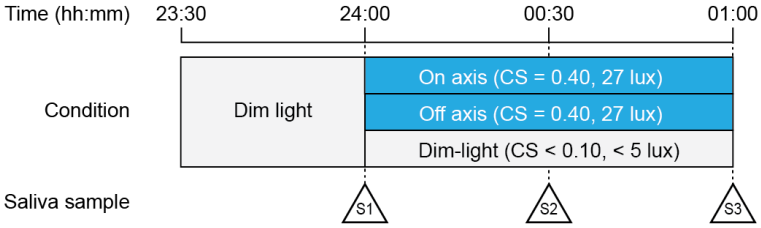

Fig. 5. Experimental protocol showing the relationship between on-axis and off-axis conditions, and salivary melatonin sample times (S1 [0 min], S2 [30 min], and S3 [60 min]).

Over the course of each experimental session, 3 saliva samples (S1, S2, S3) will be collected from each participant for melatonin analysis. Saliva samples (1 ml) will be collected using the Salivette system (Sarstedt, Nümbrecht, DE), wherein the participant chews on a plain cotton cylinder for 1-2 min, which is immediately placed in a test tube, centrifuged for 5 min at 1000 g, and frozen (-20 ° C). The frozen samples from each session will be assayed in a single batch using melatonin radioimmunoassay kits (Direct Melatonin RIA, ALPCO, Salem, NH, USA).

Sample Size and Estimated Power. The study will have enough power to detect treatment differences. Previous work by our lab[74] provides measures of variability for our primary outcome measures (melatonin suppression and questionnaires). In particular, a prior within subjects' study[74] involving effects of white light on nighttime melatonin suppression have shown that a sample size of 12 or more participants is enough to detect statistically significant (alpha = 0.05) difference in suppression (≈ 14%) for a large effect (Cohen's d > 0.5) at a statistical power ≈ 95%. A sample size of 19 subjects (24 subjects, 20% attrition) for each group, therefore, should be sufficient for the detection of significant differences in melatonin suppression.

Data and Statistical Analyses

Anticipated Outcomes. Our hypothesis is that greater melatonin suppression will be observed for the on-axis interventions, compared to the off-axis interventions, due to 2 reasons: (1) the greater density of cone photoreceptors in central retina (which will account for about 12% melatonin suppression difference according to CS model calculations [70]) and (2) the *Stiles-Crawford* effect, which suggests that the most effective optical rays incident on the retina are along the foveal axis. Overall, we also expect to see greater melatonin suppression for the low MPOD group compared to the high MPOD group, as higher MPOD means greater attenuation of the photic stimulus. We further hypothesize a significant interaction between the effect of MPOD and light source spectrum, as the yellow macular pigment preferentially filters out short-wavelength radiation, and hence should only attenuate the blue light stimulus. In other words, for high MPOD subjects, we expect to see greater melatonin suppression following exposure to the green light as compared to the blue light exposure. Lastly, we expect to observe a significant interaction between the effect of MPOD and lighting distribution, as since the yellow macular pigment is located primarily in the central retina, the off-axis should dose should not be affected by it. Finally, we hypothesize that lower MPOD scores will be associated with lower cognition.

Overall Analyses will include (1) evaluation to determine data quality, (2) creation of constructed variables and determination of variable distributions, (3) applying tests of parametric statistical assumptions wherever appropriate, (4) preliminary (generally bivariate) testing of hypotheses, (5) consideration of potential covariates to build into the statistical models to increase the probability of detecting treatment effects if they exist, and (6) development and elaboration of multivariate models. SPSS software (V25, IBM, Armonk, NY, USA) will be used for all statistical analyses (significance, p <0.05). Dr. Nagare will perform all statistical analyses and work with Drs. Figueiro and Rea on statistical analyses.

Aims 1, 2: In order to evaluate outcomes of the intervention, we will first determine the melatonin suppression for each condition by comparing melatonin levels collected during the dim-light control week to those at the same time in the experimental conditions' weeks. We will then perform a mixed-model analysis of variance (ANOVA), with lighting distribution (on-axis vs. off-axis) and spectrum (blue light vs. green light) serving as the within-subjects factors, and MPOD group (high MPOD > 0.5 vs. low MPOD < 0.3) and cognitive status (MOCA scores) (healthy vs. MCI participants) serving as the between-subjects factors. Mixed model analyses allow for different numbers of observations for different subjects (e.g. if there are dropouts). As sample size and its associated degrees of freedom allow, we will also adjust for covariates that may be associated with outcome measures, such as retinal illuminance, gender, season and any medication load. Within the limits of the sample, we will explore treatment-covariate interactions for each participant-level covariate. If strong interactions are observed, it will suggest that a future study could possibly be stratified on the given factor. To further assess the impact of MPOD on individual light sensitivity, a Pearson correlation coefficient will be calculated between melatonin suppression and MPOD values recorded across MCI patients and healthy older adults. We hypothesize a negative correlation, wherein higher MPOD values will be associated with lower suppression.

Aim 3: To investigate an association between MPOD and cognition, a Pearson correlation coefficient will also be calculated between MPOD and MoCA scores and the cognitive tests scores. We hypothesize a positive correlation, wherein lower MPOD values will be associated with lower cognition.

Potential pitfalls and Alternate Approaches . Experiment validity will be assessed by observing the expected main effect of light exposure duration, wherein we should be recording greater melatonin suppression following 60-min exposure as compared to the 30-min exposure. In case, the experiment results fail to report significant main effects due to chromatic adaptation to the narrowband light exposures, as an alternative approach, the study would be re-run using polychromatic white light sources, at the expense of not examining the spectrum effect.

Timeline: Detailed in human subjects' section.

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