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Melatonin suppression by light involves different retinal photoreceptors in young and older adults

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Abstract

Age-related sleep and circadian rhythm disturbances may be due to altered nonvisual photoreception. Here, we investigated the temporal dynamics of light-induced melatonin suppression in young and older individuals. In a within-subject design study, young and older participants were exposed for 60 min (0030-0130 at night) to nine narrow-band lights (range: 420–620 nm). Plasma melatonin suppression was calculated at 15, 30, 45, and 60 min time intervals. Individual spectral sensitivity of melatonin suppression and photoreceptor contribution were predicted for each interval and age group. In young participants, melanopsin solely drove melatonin suppression at all time intervals, with a peak sensitivity at 485.3 nm established only after 15 min of light exposure. Conversely, in older participants, spectral light-driven melatonin suppression was best explained by a more complex model combining melanopsin, S-cone, and M-cone functions, with a stable peak (~500 nm) at 30, 45, and 60 min of light exposure. Aging is associated with a distinct photoreceptor contribution to melatonin suppression by light. While in young adults melanopsin-only photoreception is a reliable predictor of melatonin suppression, in older individuals this process is jointly driven by melanopsin, S-cone, and M-cone functions. These findings offer new prospects for customizing light therapy for older individuals.

KEYWORDS

aging, circadian, mathematical modeling, melanopsin, melatonin, photoreception, sleep

Raymond P. Najjar and Abhishek S. Prayag contributed equally to this work.

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1 | INTRODUCTION

In the retina, a small subset of intrinsically-photosensitive retinal ganglion cells (ipRGCs), with a peak sensitivity to blue light (λ_{\max} ~480 nm),^{1,2} integrate phototransduced light signals from rods (λ_{\max} ~505 nm), and cones (S-cones, λ_{\max} ~420 nm; M-cones, λ_{\max} ~530 nm; L-cones, λ_{\max} ~558 nm),^{3,4} before projecting to the suprachiasmatic nucleus (SCN) and other nonvisual brain areas.⁵⁻⁷ This set-up oversees the spectro-temporal sensitivity of various nonvisual responses to light including the pupillary light response (PLR), melatonin suppression, and circadian entrainment.

In mammals, including humans, ipRGCs integrate the input from cones and rods in an additive (ON) or opponent (OFF) manner.^{2,8-11} In nonhuman primate retinas, single unit recordings of ipRGCs highlighted that melanopsin-based signaling is combined with a convergent ON input from L- and M-cones, and an OFF input from S-cones in response to 550 nm light.² Similarly, in humans, intricate combinations of photoreceptor signaling (melanopsin, S-, M-, L-cones, hereafter described as S, M, and L) have been shown to regulate the PLR and other nonvisual responses to light.⁸⁻¹¹ For instance, Spitschan et al. suggested that the PLR is governed by an ON contribution from melanopsin and L + M; but an OFF contribution from S.⁹ Compared to the PLR, recent findings from our group and others suggest that melanopsin may fully account for melatonin suppression by light.^{10,12,13} Accordingly, a recent analytical paper by Giménez et al. highlighted a sigmoidal dose-response relationship between melatonin suppression and melanopic equivalent daylight illuminance, but not with photopic illuminance.¹⁴ While all of the aforementioned studies focused on healthy young individuals, to date, the impact of aging on photoreceptor contribution to nonvisual responses to light in general, and melatonin suppression in particular, has not been investigated.

Aging is associated with a host of changes, in sleep and circadian physiology, in neuronal and behavioral plasticity, all of which contribute to the neurobehavioral alterations observed in the elderly.¹⁵⁻²² These changes could be due, in part, to age-related alterations of the circadian timing system,^{16,23,24} either at the eye level,²⁵⁻²⁷ at the central clock level in the SCN,²⁸⁻³¹ or at output levels.³²⁻³⁵ While others have reported reduced melatonin suppression by white³⁶ and short wavelength light³⁷ in older participants, our team showed no reduction in suppression in response to 60 min of light exposure, but revealed a shift in the peak spectral sensitivity of melatonin suppression to longer wavelength lights in the elderly compared to young.²⁵ Our findings suggested a reshuffling of the photoreceptor contribution to melatonin suppression with aging.

In this study, we investigated the temporal dynamics of melatonin suppression in response to light at night, in young and older participants, at the finer timescale of 15 min intervals. In addition, we further addressed photoreceptor contribution to melatonin suppression by light over time in both age groups.

2 | MATERIALS AND METHODS

2.1 | Participants

Thirteen healthy participants were recruited in a within subject design study (130 light exposure sessions overall, see details below) via local advertisement. Participants were assigned into two age groups, a younger group including five participants (five men, 25.8 ± 0.73 years, mean \pm SE), and an older group including eight participants (two men and six women, 59.4 ± 0.99 years, mean \pm SE). The screening procedure included a comprehensive ophthalmologic examination by a collaborating ophthalmologist that included slit lamp examination, standard automated perimetry (Humphrey Field Analyzer™, Carl Zeiss Meditec; Sita-standard 24-2), and color vision tests (Farnsworth-Munsell 100 Hue test) to exclude patients with ocular diseases. All participants were phakic in both eyes, nonsmokers, nonalcoholic and free of medications known to affect sleep, the visual and circadian systems, and melatonin secretion. All participants were in good mental health (mini mental score > 28) and had no sleep or psychiatric disorders as revealed by the Pittsburgh sleep quality index, the SIGH SAD, and the PIDS-SA questionnaires. None of the participants was found to be an extreme morning or evening type by the Horne-Östberg morningness-eveningness questionnaire. Participants were asked to maintain a regular sleep wake cycle during and 3 weeks before study, and their rest activity pattern was verified by wrist actimetry (Actitrac™; IM Systems). Participants' sleep stability was also verified throughout the study using sleep diaries.

Before participation, participants provided written informed consent after receiving a detailed description of the procedures and purpose of the experiments. All procedures were in compliance with the institutional guidelines and the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board.

2.2 | Experimental protocol

Each participant underwent 10 experimental night sessions (nine light sessions and one control session) scheduled between 1930 and 0400 (see Najjar et al.²⁵ for

more details). During each session, participants were exposed for 60 min to one of nine monochromatic light stimuli (peak wavelengths: 420–620 nm). Conversely, participants were not exposed to light during the dark control session, to assess their individual endogenous melatonin profile. Nevertheless, during the control session, participants underwent the same experimental procedures (e.g., posture, sitting in front of the Ganzfeld sphere, blood sampling, etc.), except light exposure, as during the nine light sessions. The order of the sessions was randomized for each participant, and sessions were separated by a minimum of 1 week, to disperse any potential phase shifting effect of light on the circadian system from one session to another. Participants were maintained in an ambient very dim light (<1 lux) between 2000 and 2200 to measure their unmasked dim light melatonin onset (DLMO) and in complete darkness (obscure room and blindfolded) from 2200 to 0030 and 0130 to 0400. During that period of time, participants were in semi-recumbent posture (45°) in their bed to avoid possible effects of postural changes on melatonin concentration.³⁸ Light exposure was scheduled between 0030 and 0130 for all subjects. During the light stimulation, participants were in a sitting position (90°). Pupils were dilated using tropicamide (2 mg/0.4 mL; Novartis Ophthalmics), with one drop repeated three times before light exposure (−60, −45, and −30 min). Participants were not allowed to use their phones or perform any visual tasks requiring light (reading, watching television, etc.) throughout the session but were allowed to sleep before and after the light stimulation. Fluid intake was controlled to avoid the dilution of melatonin concentrations in blood samples.

Blood samples (4 mL) were collected in a heparinized plastic tube via an indwelling catheter at different time intervals during the session (every 60 min from 2000 to 2200 and 0300 to 0400, 30 min from 2000 to 2330 and 0200 to 0300, and 15 min between 2330 and 0200). Following the blood sample taken at 0030, four blood samples were taken during the light exposure (or in darkness for the control session) at 15, 30, 45, and 60 min after 0030 (i.e., light onset time or in darkness for the control session). Samples were then stored at 4°C and centrifuged at the end of the session (2000g for 20 min). Plasma was decanted and stored at −20°C until assayed. All experimental sessions were performed at the Chronobiology Platform (Edouard Herriot Hospital).

2.3 | Light exposures

A white tungsten halogen light (3250 K) was collimated through monochromatic filters (full width at half

maximum = ~10 nm, omega optics, λ_{\max} 420, 440, 460, 480, 500, 530, 560, 590, and 620 nm). After diffusion by an opaline filter, light was projected into a Ganzfeld sphere with a diameter of 45 cm, coated with white high reflectance paint. The sphere allowed a constant and uniform illumination of the entire retina. The participant's head was maintained in a constant position inside the sphere by an ophthalmologic head holder. Light exposures were calibrated to be of equal photon density (3.16×10^{13} photons/cm²/s) at the subjects' eye level using a radiometer (International Light® IL 1700). The spectral emission characteristics of the lights were verified using a spectrophotometer (Ocean Optics USB4000; Ocean Optics). Gaze behavior and pupil dilation were monitored by the experimenter to ensure constancy of exposure for all conditions.

2.4 | Melatonin radioimmunoassay (RIA)

For technical reasons, two RIA were used in the study. Out of 13 subjects, the samples of seven participants (five young and two older) were assayed using a radio-immunological method developed by Claustrat et al.³⁹ Melatonin concentrations were determined in duplicate after diethylether extraction. The assay used an iodinated ligand. Functional sensitivity was 2.6 pg/mL. Inter-assay coefficient of variation was 11% at 50 pg/mL and 13% at 100 pg/mL ($n = 15$). Intra-assay coefficient of variation was 7% at 50 pg/mL and 9% at 100 pg/mL ($n = 12$).

The samples of the remaining six subjects were assayed using the Bühlmann melatonin RIA kit (Bühlmann AG). With this assay, melatonin concentrations were determined in duplicate after C18 solid phase extraction by a double antibody RIA based on the Kennaway G280 anti-melatonin antibody.⁴⁰ Analytical sensitivity was 0.3 pg/mL and functional sensitivity 0.9 pg/mL. Inter-assay coefficient of variation was 18.1% at 1.9 pg/mL and 10.1% at 24 pg/mL ($n = 18$ samples). Intra-assay coefficient of variation was 9.4% at 1.9 pg/mL and 6% at 24 pg/mL ($n = 18$ samples).

The resulting concentrations from the two assays were highly correlated ($n = 15$ samples from one participant; $r = .90$, $p < .001$).

2.5 | Data and statistical analysis

2.5.1 | Melatonin secretion and suppression

Individual melatonin amplitude and DLMO were calculated as the maximal secretion of the smoothed

melatonin profiles (locally weighted scatterplot smoothing LOESS method, span = 0.5) and the 25% upward crossing (100% being the melatonin amplitude calculated between the 0030 and the 2000 concentrations), respectively. DLMOs were compared across sessions and between subjects using a two-way analysis of variance (ANOVA) for repeated measures (within: time; between: subject). DLMO were compared between young and aged groups using a two-way ANOVA for repeated measures with (within: wavelength; between: groups). Melatonin secretion amplitudes assessed during the control session were compared between groups using a Mann–Whitney rank sum test.

The percentage of melatonin suppression was calculated for each participant's light and control sessions at four time points during the session (i.e., 15, 30, 45, and 60 min after session onset which occurred at 0030). Below is an example of percentage melatonin suppression calculated 60 min after light onset (i.e., at 0130) (Equation 1).

$$\begin{aligned} \% \text{ Mel Supp} &= -100 \\ &\quad \frac{\text{0130 mel concentration}}{\text{0030 mel concentration}} \\ &\times \frac{-\text{0030 mel concentration}}{\text{0030 mel concentration}} \end{aligned} \quad (1)$$

To account for individual differences in melatonin secretion profiles that are independent of light,^{41,42} the percent melatonin suppression score was then normalized to the control session (%CA) at respective time points (Equation 2).

$$\begin{aligned} \text{Mel Supp (\%CA)} &= \% \text{ Mel Supp (light exposure session)} \\ &\quad - \% \text{ Mel Supp (control session)} \end{aligned} \quad (2)$$

2.6 | Modeling the photoreceptor contribution to melatonin suppression

We used two approaches to model retinal photoreceptors' contribution to melatonin suppression in both age groups.

Approach 1: Given that melanopsin strongly predicts melatonin suppression by light in the young,^{12,13} we first modeled a melanopsin-only contribution to melatonin suppression in the young and older groups, at each of the four time points of light exposure (15, 30, 45, and 60 min after light onset). The Govardovskii alpha-band template⁴³ was used to generate opsin templates with constrained peak sensitivity. A melanopsin sensitivity function was thus fitted on the average, control-adjusted,

percentage melatonin suppression, measured at each time interval and for each group (Equation 3).

$$\begin{aligned} &\text{Melatonin suppression} \\ &\quad \frac{a}{\exp[A(a-x)] + \exp[B(b-x)]} \\ &\quad + \exp[C(c-x)] + D \end{aligned} \quad (3)$$

where $x = 480/\text{wavelength}$. Other parameter values were $A = 69.7$, $a = 0.88$, $B = 28$, $b = 0.922$, $C = -14.9$, $c = 1.104$, $D = 0.674$ as in the Govardovskii et al.⁴³

The amplitude, a , was not constrained to the value of 1 as in Govardovskii et al.,⁴³ but allowed to be optimally determined by the nonlinear least-squares curve-fitting procedure. The peak sensitivity of melanopsin was fixed at 480 nm.⁴⁴

Given that the data set used is identical to the previously published data in Najjar et al.,²⁵ the average lens filtering spectra obtained for the young and the older in that study was applied so as to account for the lens transmittance and its change with aging. Each fitted function in the young and older participants was accounted for prereceptor filtering by multiplying the function with its respective ocular lens transmittance spectra, published in Najjar et al.²⁵ Peak spectral sensitivities (λ_{max}) for both groups (young, older), at the four time-intervals (15, 30, 45, 60 min) following light onset, were predicted using this melanopsin nomogram corrected for the respective transmittance of the ocular media in younger and older participants. The correlation coefficients (R^2 and adjusted- R^2) were retrieved as a measure of the goodness of each model and for comparison with coefficients previously reported in other studies. The adjusted- R^2 accounted for the number of predictors in each model. The fitting of the curves was carried out using nonlinear least squares fitting procedures in R v. 3.6.2 statistical environment (<http://cran.r-project.org>).

Approach 2: We then evaluated the combined contribution of melanopsin and cone photoreceptors in the melatonin suppression using a backward stepwise selection approach.⁴⁵ First, the Govardovskii alpha-band template⁴³ was used to fit melanopsin as well as S-, M-, and L-cone opsin nomograms on the average, control-adjusted, percentage melatonin suppression, measured at each of the four time-intervals of light exposure in the young and older groups. The amplitudes assigned to each photoreceptor, a_1 , a_2 , a_3 , a_4 , were not constrained but allowed to be optimally determined by the nonlinear least-squares curve-fitting procedure (Equation 4).

Peak sensitivities were kept at 420 nm for S-opsin (S-cone), 530 nm for M-opsin (M-cone), and 558 nm for

L-opsin (L-cone).⁴⁴ Melanopsin sensitivity was fixed at 480 nm.⁴⁴

$$\begin{aligned} & \text{Melatonin suppression} \\ & \sim \frac{a1}{\exp[A(a-x1)] + \exp[B(b-x1)]} \\ & \quad + \exp[C(c-x1)] + D \\ & + \frac{a2}{\exp[A(a-x2)] + \exp[B(b-x2)]} \\ & \quad + \exp[C(c-x2)] + D \\ & + \dots \dots \dots \end{aligned} \quad (4)$$

where $x1 = 480/\text{wavelength}$, $x2 = 420/\text{wavelength}$, and so on. Other parameter values are the same as in Equation 3.

Thus for a particular time interval, melanopsin nomogram with amplitude $a1$, and cone opsin nomograms (S-cone, M-cone, L-cone, with amplitude $a2$, $a3$, $a4$, respectively) were simultaneously fitted on the melatonin suppression data. Melanopsin, S-, M-, and L-cone contribution were not constrained; their amplitude could be either positive or negative, as determined by the modeling procedure. As in the first approach, we corrected for ocular media transmittance. The two lens filtering spectra obtained in the younger and older age groups²⁵ were applied as a multiplicative term to each sensitivity functions.

We then explored photoreceptor (melanopsin, S-cone, M-cone, L-cone) contributions in our melatonin suppression data. The amplitude parameter for each photoreceptor nomogram ($a1$, $a2$, $a3$, $a4$) was estimated by the nonlinear least-squares regression model in R. The p -value of the t -test carried out to predict the significance of each amplitude parameter was determined, with significant level set at $p < .05$. The least significant parameter was dropped and the model refitted at each successive step, in a backward stepwise selection approach.⁴⁵ Thus, we began an iterative process whereby the photoreceptor showing the poorest nonsignificant contribution was iteratively dropped from the initial four photoreceptor model, until significant photopigment contribution(s) was detected, if any (see Supporting Information S1: Tables 1 and 2). We thus evaluated the photopigment(s) contributing to melatonin suppression by light for each group (young, older) and at each time interval (15, 30, 45, 60 min), to propose a model for the dynamics of melatonin suppression in the young and the older, at each respective duration of light exposure. If more than one photoreceptor was involved (e.g., +melanopsin + S-cone + L-cone) the fitted function was thus a mathematical combination of the photoreceptor nomograms,

accounted for prereceptor filtering by multiplying the function with its respective ocular lens transmittance spectra.²⁵ We assessed the assumptions of normality (Shapiro–Wilk's test), homogeneity (Levene's test), and independence (lag plot) of the residuals to validate each optimal model.⁴⁶ These assumptions were not violated, validating the predicted optimal models of photoreceptor contribution at each time interval and for each age group.

3 | RESULTS

3.1 | Amplitude and phase of melatonin secretion in both groups

All participants displayed a significant nocturnal secretion of melatonin. The amplitudes of melatonin secretion assessed during the control session, were not different between young (65.8 ± 20.9 pg/mL) and older participants (89.3 ± 27.3 pg/mL) ($p = .5$). DLMOs, as markers of circadian phases, were not different between groups ($F(1, 11) = 1.36$, $p = .26$) and did not vary across night sessions ($F(9, 99) = 0.43$, $p = .92$). Mean DLMO was 2201 ± 0027 h in the young and 2228 ± 0028 h in the older participants.

3.2 | Melanopsin alone drives melatonin suppression by light in young but not older participants

Given that melanopsin is the predominant driver of melatonin suppression,¹³ we evaluated melanopsin-only contribution in the response levels we observed. Our approach was further supported by recent results demonstrating that melanopsin fully accounts for the different nonvisual responses observed across a wide range of light stimuli.^{12,14} In young participants, the fitted melanopsin-only nomograms at the four time intervals yielded adjusted R^2 values ranging between 0.62 and 0.94 (Figure 1A–D and Table 1). In older participants, adjusted R^2 values were systematically lower compared to those in the young, ranging from -0.59 to 0.59 (Figure 1E–H and Table 1). The negative adjusted R^2 value (-0.59) at 15 min in the older is indicative of a poor regression and is therefore not an appropriate model of melatonin suppression. Peak melatonin suppression was at 485.3 nm at all time intervals in the young group (Figure 2A and Table 1). In the older group, peak melatonin suppression was at 495.3 nm at all time intervals (Figure 2B and Table 1). These disparities between the two groups, that is, the shift in toward the green part of the visible spectrum by 10 nm in the older

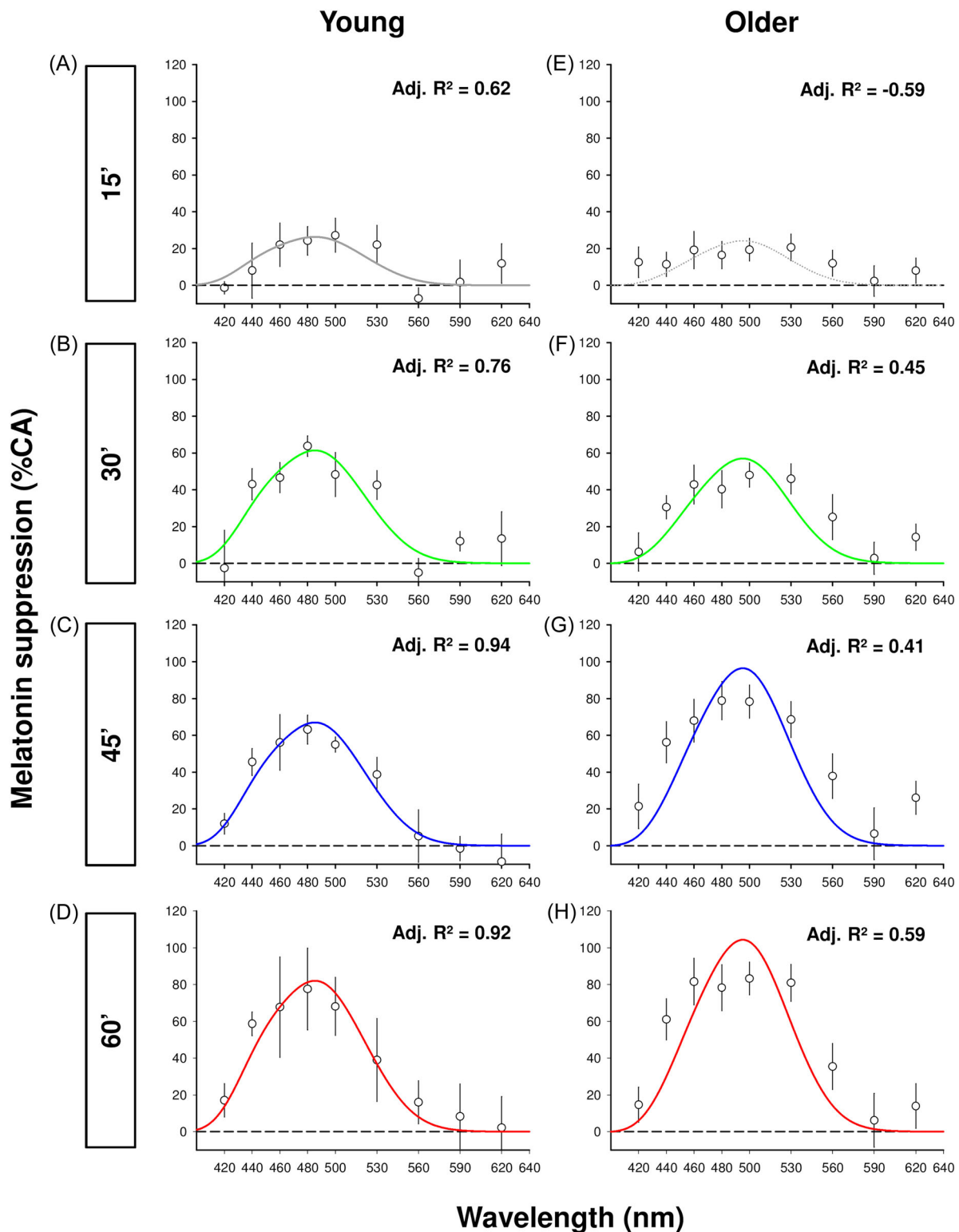


FIGURE 1 Approach 1 The dynamic spectral sensitivity of melatonin suppression by light in young and older participants, fitted with a melatonin-only model. (A) Melatonin sensitivity function was fitted on the average, control-adjusted, percentage melatonin suppression, measured at each time interval for the young (A–D) and older (E–H) participants. Quantified melatonin suppression values are shown in open circle as average \pm SE. Each melatonin-only nomogram was corrected for the respective transmittance of the crystalline lens. The adjusted R^2 of the model at 15 min in older participants was negative, indicating a poor/unreliable fit represented by a dotted gray line. The adjusted R^2 for each fitted functions is given as a measure of the goodness of fit and for comparison with literature.

group, as well as the poorer R^2 values, are consistent with a multiopsin contribution to melatonin suppression by light in the older participants.

3.3 | Melanopsin + S-cone + M-cone drive melatonin suppression in older participants after 30 min of light exposure

We evaluated the combination of the four retinal photoreceptors (melanopsin, S-cone, M-cone, L-cone) driving the melatonin suppression response. Beginning with the model containing all four photoreceptor functions, we started dropping the least significant photoreceptor contribution for each group and each interval (Supporting Information S1: Tables 1–2) until significant contribution (s) were observed, if any. The best predictor of melatonin

suppression in the young group, at all of the time intervals, was melanopsin. Peak melatonin suppression remained stable at 485.3 nm in the young group across all time intervals (Figure 4A). Given that melanopsin is the best predictor in the young using this backward stepwise selection approach, the melanopsin fit, λ_{\max} and R^2 are identical (Figure 3A–D and Table 2) to the melanopsin-only function applied approach 1 (Figure 1A–D and Table 1). Taken together, both results confirm the melanopsin function as the best fit in our young group data. Conversely, in the older group, the model that fitted our data best was the +melanopsin + S-cone + M-cone model, at the 30, 45, 60 min time intervals (Figure 3E–H and Supporting Information S1: Table 2) with respective adjusted R^2 values of 0.83, 0.82, and 0.92 (Table 2). At these time intervals, R^2 values of the +melanopsin + S-cone + M-cone model were higher than those of the melanopsin-only model (Table 2 vs. Table 1), indicating better modeling of the suppression by the +melanopsin + S-cone + M-cone model in the older group (Figures 3F–H and 4B). After 15 min of light exposure, however, the spectral melatonin suppression data were best, albeit weakly, fitted with a +S-cone + M-cone model ($R^2 = 0.32$, adjusted $R^2 = 0.09$) with two distinct peaks at 450.5 and 538.9 nm, without any significant melanopsin contribution (Figures 3E and 4B, Table 2). Conversely, in the older group, the data at 30, 45, and 60 min intervals, best fitted with the +melanopsin + S-cone + M-cone model, yielded peaks at 503.5 nm (30 min), 498.4 nm (45 min), and 497.3 (60 min) (Figures 3F–H and 4B, Table 2).

TABLE 1 Approach 1—Peak spectral sensitivity throughout the light exposure using a melanopsin-only model.

Time	Young			Older		
	λ_{\max} (nm)	R^2	Adj. R^2	λ_{\max} (nm)	R^2	Adj. R^2
15'	485.3	0.66	0.62	495.3	−0.39	−0.59
30'	485.3	0.79	0.76	495.3	0.52	0.45
45'	485.3	0.95	0.94	495.3	0.48	0.41
60'	485.3	0.93	0.92	495.3	0.64	0.59

Note: Predicted peak spectral sensitivity using the melanopsin-only model (Approach 1) at intervals of 15 min in young and older participants.

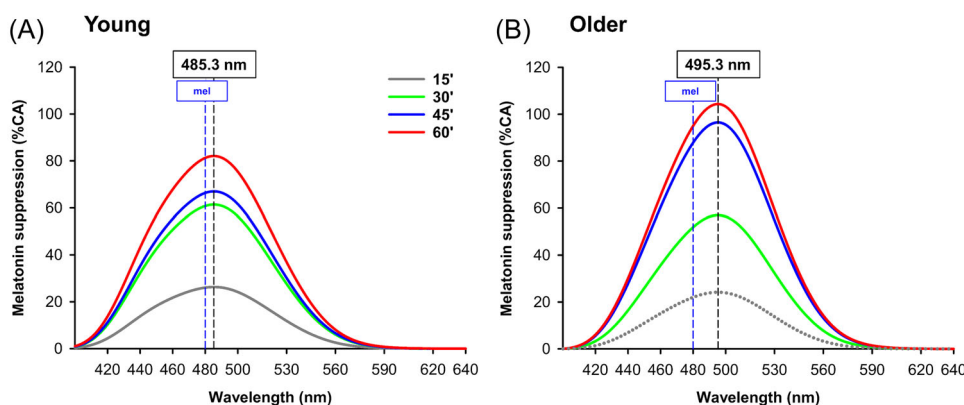


FIGURE 2 Approach 1 Overlaid spectral sensitivity curves (melanopsin-only model) for melatonin suppression by light in young and older participants throughout the 60 min light exposure. Overlaid spectral sensitivity curves (corrected for the respective transmittance of the lens) obtained in Figure 1. (A) The melanopsin-only fitted function predicts a constant peak spectral sensitivity at 485.3 nm (dashed vertical line) in young participants over time, at 15, 30, 45, and 60 min of light exposure. (B) The melanopsin-only fitted function predicts a constant peak spectral sensitivity different from the young by 10 nm, at ~495.3 nm (dashed vertical line) in older participants after 15, 30, 45, and 60 min of light exposure. The adjusted R^2 at 15 min in older participants was negative, indicating a poor/unreliable fit represented by a dotted gray line. The dashed blue vertical line (mel) at 480 nm represents the peak spectral sensitivity of melanopsin before lens density correction. The predicted peak in each group and R^2 at each 15 min interval are provided in Table 1.

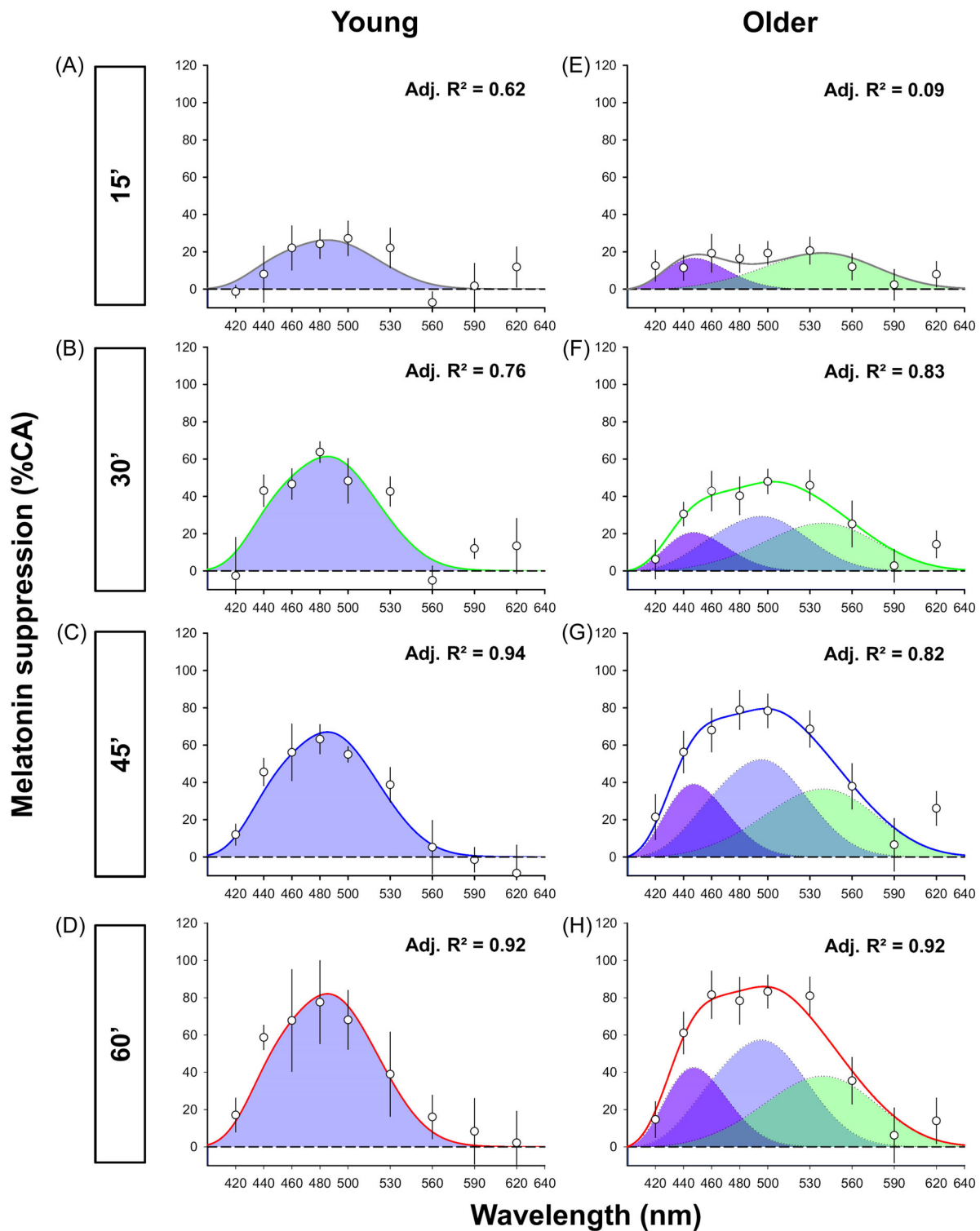


FIGURE 3 Approach 2 The dynamic spectral sensitivity of melatonin suppression by light in young and older participants fitted with a composite model. (A–D) Following this backward stepwise selection approach, spectral melatonin suppression by light in the young was best predicted by a melanopsin-only model at the 15, 30, 45, and 60 min time points of the light exposure. As expected, the peak spectral sensitivity and R^2 values were identical to the melanopsin-only function fitted in Figure 1. (E) In older participants, our backward stepwise selection approach predicted significant +S-cone (purple shaded area) + M-cone (green shaded area) contribution to the spectral melatonin suppression response at 15 min of the light exposure. Subsequently, at 30, 45, and 60 min the model with +melanopsin (blue shaded area) + S-cone + M-cone best fitted the melatonin suppression data in the elderly (F–H). Quantified melatonin suppression values are shown in open circle as average \pm SE. Each nomogram was corrected for the respective transmittance of the crystalline lens. The adjusted R^2 for each fitted functions is given as a measure of the goodness of fit and for comparison with literature.

4 | DISCUSSION

Our results show that although melatonin suppression by light is predominantly, if not solely, driven by melanopsin in young individuals, a melanopsin-based model is insufficient to characterize melatonin suppression by light in older individuals. While melanopsin input is not

TABLE 2 Approach 2—Peak spectral sensitivity throughout the light exposure using a composite model approach in the young and old groups.

Time	Young			Older		
	λ_{\max} (nm)	R^2	Adj. R^2	λ_{\max} (nm)	R^2	Adj. R^2
15'	485.3	0.66	0.62	450.5; 538.9	0.32	0.09
30'	485.3	0.79	0.76	503.5	0.89	0.83
45'	485.3	0.95	0.94	498.4	0.89	0.82
60'	485.3	0.93	0.92	497.3	0.95	0.92

Note: In the young group, following backward stepwise selection approach (Approach 2), spectral melatonin suppression by light in the young was best predicted by a melanopsin-only model at the 15, 30, 45, and 60 min time points of the light exposure, with adj. R^2 ranging from 0.62 to 0.94. In the older group, a +S-cone + M-cone model best fitted the data at 15 min whereas a +melanopsin + S-cone + M-cone model best fitted the data at 30, 45, and 60 min of light exposure. Higher R^2 and adjusted R^2 values compared to Table 1 indicate better prediction of melatonin suppression by the composite models in older participants.

significant in the elderly at 15 min of light exposure, it is coupled with the positive contribution of S-cones and M-cones from 30 min onwards, leading to a shift in peak nonvisual spectral sensitivity from 485 nm in the young to an average peak around 500 nm across the light exposure in the elderly.

In young individuals, our model estimated a peak for melatonin suppression around 485 nm after 15 min of monochromatic light exposure. This peak remained constant throughout the 60 min light exposure. This spectral sensitivity of melatonin suppression is close to the 480 nm peak sensitivity of melanopsin^{1,2} and therefore supports the predominant role of melanopsin in melatonin suppression during the 60 min light exposure in the young.^{12,13} On the other hand, in older individuals, 15 min of exposure to narrow-band light was not sustained by any melanopsin contribution and spectral melatonin suppression was predominantly driven by +S-cone + M-cone model. Hypothetically, this may be due to either a decreased/sluggish melanopsin contribution to melatonin suppression in the elderly and/or increased cone contribution in shorter and longer wavelengths early in the light exposure. Note that the fitted model had, however, a low adjusted R^2 , and these findings should be investigated further. Conversely, the peak spectral sensitivity of melatonin suppression established after 30 min of light exposure was then relatively stable, around 500 nm, across

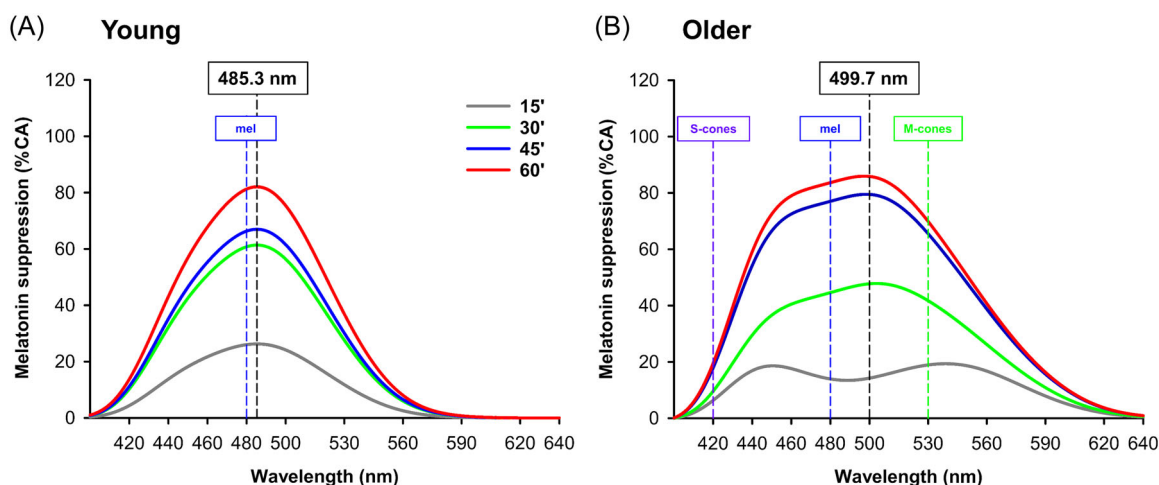


FIGURE 4 Approach 2 Overlaid spectral sensitivity curves (composite model) for melatonin suppression by light in young and older participants throughout the 60 min light exposure. Overlaid spectral sensitivity curves (corrected for the respective transmittance of the lens) obtained in Figure 3. (A) In young participants, spectral melatonin suppression was robustly predicted using a melanopsin-only model at 15, 30, 45, and 60 min intervals respectively. Dashed vertical lines at 485.3 nm represent the constant peak spectral sensitivity over time in the young group. (B) In older participants, spectral melatonin suppression was better predicted by a composite +S-cone + M-cone model at 15 min and +melanopsin + S-cone + M-cone model at the 30, 45, and 60 min time points of the light exposure. Dashed vertical line at 499.7 nm represent the predicted average peak spectral sensitivity over time of the combined +melanopsin + S-cone + M-cone function in the older group. The predicted peak in each group and R^2 at each 15 min interval are given in Table 2. The dashed vertical lines at 420 nm (purple), 480 nm (blue), and 530 nm (green) represent the peak spectral sensitivity of S-cones, melanopsin, and M-cones, respectively, before lens density correction.

the remaining 30 min of the light exposure. These findings suggest that additional opsins, besides melatonin, are involved in melatonin suppression in older individuals. A robust contribution of +M-cones to melatonin suppression could explain the shift in peak spectral sensitivity from ~485 nm in the young to ~500 nm in the older. This is in accordance with data showing excitatory L + M-cone ON-input to ipRGCs in primates.² A similar additive input of melanopsin and longer wavelengths (L-cones) has also been observed for the human PLR.^{9,11,47} Conversely, Spitschan et al.,⁹ and Woelder' et al., findings highlight a negative S-cone and negative M-cone contribution to the PLR, albeit in younger individuals. The divergence in our findings underscores the possibility that each nonvisual response might be influenced by specific photoreceptor integration models.

Here, we suggest that retinal photoreceptor adaptation, consistent with the reorganization that the human retinal network undergoes with aging,⁴⁸ is one possible mechanism behind the differences in photoreceptor contribution between young and older group. They might also result from adaptation mechanisms of the nonvisual response per se. For instance, Giménez et al.,⁴⁹ showed that melatonin suppression was lower in subjects wearing blue-blocking contact lenses during a 30 min light exposure. However, after 16 days of continuously wearing blue-blocking lens, melatonin suppression was not different anymore from the control condition (normal contact lenses) in young healthy adults. These findings, suggest that nonvisual photoreception is plastic and can undergo quick adaptations/compensations in young individuals. Similar compensatory mechanisms can also occur in older individuals who have less blue light reaching the retina due to ocular lens yellowing.^{26,50,51} In fact, Najjar et al.,²⁵ have shown that although lens yellowing leads to a 42% decrease in short-wavelength light reaching the retina in the older individuals, this was not associated with an attenuation of melatonin suppression to short-wavelength lights. Taken together, retinal restructuring and reorganization coupled with potential adaptation/compensatory mechanisms,^{48,52,53} could explain age-related changes in photoreceptor contribution to nonvisual responses shown in our study.

Findings from this study confirm the peak spectral sensitivities observed by Najjar et al.,²⁵ following 60 min of light in young and old participants and corroborate melanopsin stimulation by light as the predominant predictor of nonvisual responses.^{12–14} Of note, Brown¹² recently observed that the degree melanopsin stimulation, calculated via melanopic illuminances, accurately predicts the response levels of melatonin suppression and circadian phase resetting, across an array of lighting

types, light spectra, and exposure durations. In that study, analysis was restricted to an age range of 20–45 years, which would therefore include our young group (mean age of 25.8 years) but not our old group (mean age of 59.4 years). Consistent with Brown,¹² our results in young participants confirm the observation that melanopsin can solely account for melatonin suppression. They are also consistent with results by Prayag et al.,¹³ which show that melanopsin is the predominant contributor to melatonin suppression in response to a 90 min of monochromatic light exposure in an equivalent young age group (mean age of 24.5 years). Our results in the older individuals do not invalidate the conclusions of these^{12,13} but indicate that the relative contribution of retinal photoreceptors is dependent on the nonvisual response measured, the duration and irradiance of the light exposure and is likely to vary with age.

While, to our knowledge, we are the first to report a nonvisual spectral sensitivity curve and consequently photoreceptor contribution in older participants, we also report that melanopsin photoreception can fully account for the spectral melatonin suppression by light in young subjects without S-cone, M-cone, L-cone contributions within 60 min of light exposure. These findings are in agreement with Gimenez et al., showing predominant melanopsin-influence, rather than cones, at high illuminances. We also find no S-cone contribution in the young, consistent with results pointing to no evidence of S-cone involvement in melatonin suppression following differential S-cone excitation using silent substitution.¹⁰ On the other hand, our findings of “no cone contribution” to melatonin suppression in the young within the first 60 min of light exposure contrast with earlier work by Gooley et al.,⁵⁴ and St Hilaire et al.,⁵⁵ who show an increased cone contribution to melatonin suppression within the first 97.5 min of light exposure. We believe that differences between the findings are predominantly due to protocol differences (duration of the light exposure, number of wavelengths used, fixed irradiance vs. fluence response curve) and resulting analysis methods. For instance, both Gooley et al.,⁵⁴ and St Hilaire et al.,⁵⁵ quantified melatonin suppression in young participant over a much longer (i.e., 6.5 h) duration and established irradiance-response curves for blue and green monochromatic⁵⁴ or additional wavelengths.⁵⁵ Consequently, melatonin suppression and photoreceptor contribution was established at quarters of 97.5 min each which is longer than our investigation at 15, 30, 45, and 60 min. St Hilaire et al.,⁵⁵ used five narrow-band stimulations (420, 460, 480, 507, and 555 nm) whereas we utilized nine narrow-band stimulations (420, 440, 460, 480, 500, 530, 560, 590, and 620 nm), resulting in a more finely detailed spectral sensitivity

curve (i.e., four wavelengths before and four wavelengths after 500 nm). In addition, our statistical approach involved a backward stepwise selection method based on the area under the melatonin suppression curve while St Hilaire et al.,⁵⁵ used a best-fit approach. As mentioned above, the distinct S-cones contribution to melatonin suppression in the elderly may originate from compensatory mechanisms to ocular changes (e.g., lens yellowing, etc.) observed with aging.

4.1 | Limitations

Our study has a few limitations. First, the sample sizes in both groups were small ($n = 5$ in the young group, $n = 8$ in the older). While independent, single measure comparisons between both groups would have been insufficient, our protocol is a repeated-measure within-subject design which assessed each participant repeatedly, and randomly, over 10 experimental conditions, which equates to 130 individual data points. We also implemented a fitting procedure to estimate the peak sensitivity as well as a backward stepwise selection procedure to investigate photoreceptor contribution. The determination coefficients found in the two steps of our analysis, both in the young and old, are indicative of high effect sizes and statistical power, and provide confidence in the differences we observed. It is worth highlighting that adding constraints to the model based on certain, albeit not all, prior findings could influence the outcomes when employing similar analytical approaches. Second, given the proximity of the peak sensitivity between M- and L-cones (530 vs. 558 nm^{3,4}), we are unable to exclude a potential L-cones input in the suppression response in the older. This does not invalidate our results of an additive combination melanopsin, S-cone and M-cone photoreceptors in the older. Third, young and older age groups were not gender-matched. However, it's worth mentioning that all women included in our project were postmenopausal, and previous research did not report any gender-related effects on melatonin suppression by light.⁵⁶ We acknowledge, however, that we cannot definitively rule out the possibility of a gender-related effect on spectral sensitivity to light in the elderly compared to the young. Given this uncertainty, further investigations are warranted to determine whether gender plays a role in the observed spectral sensitivity differences between age groups. Finally, we also recognize that the photoreceptor contribution may yet differ across nonvisual responses to light. Thus, the photo-sensitivity predictions of our models only apply for the acute melatonin suppression response which, we further

stress, is not a reliable proxy for SCN-dependent circadian responses in humans.⁵⁷

5 | CONCLUSION

Our study highlights unexpected yet important physiological traits of the aging of nonvisual photoreception and its impact on melatonin suppression. While in young adults melanopsin-only photoreception is a reliable predictor of melatonin suppression, in older individuals this process is jointly driven by melanopsin, S- and M-cone function. These results carry an important translational value as they suggest that artificial lighting used in clinical settings, for instance for light therapy of sleep or affective disorders, or even in everyday life (e.g., blue blocking filters) may not show the same efficiency in older patients.

AUTHOR CONTRIBUTIONS

The analyses described in this article were conceived and designed by Raymond P. Najjar, Abhishek S. Prayag, and Claude Gronfier, and performed by Abhishek S. Prayag. Raymond P. Najjar, Abhishek S. Prayag, and Claude Gronfier interpreted the data and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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