INTRODUCTION

In addition to facilitating vision, ocular light exposure drives a range of neuroendocrine, physiological and behavioural responses in mammals. In humans, such responses have long been known to include resetting the circadian clock,\(^1,2\) acute suppression of pineal melatonin production,\(^3,4\) and increases in subjective and objective alertness at night and during the

Abstract

Light influences diverse aspects of human physiology and behaviour including neuroendocrine function, the circadian system and sleep. A role for melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) in driving such effects is well established. However, rod and/or cone signals routed through ipRGCs could also influence “non-visual” spectral sensitivity. In humans, this has been most extensively studied for acute, light-dependent, suppression of nocturnal melatonin production. Of the published action spectra for melatonin suppression, one demonstrates a spectral sensitivity consistent with that expected for melanopsin while our own (using briefer 30 minute light exposures) displays very high sensitivity to short wavelength light, suggesting a contribution of S-cones. To clarify that possibility, six healthy young male participants were each exposed to 30 minutes of five irradiances of 415 nm monochromatic light (1-40 µW/cm²) across different nights. These data were then combined with the original action spectrum. The aggregated data are incompatible with the involvement of any single-opsin and multi-opsin models based on the original action spectrum (including Circadian Stimulus) fail to predict the responses to 415 nm stimuli. Instead, the extended action spectrum can be most simply approximated by an ~2:1 combination of melanopsin and S-cone signals. Such a model also better describes the magnitude of melatonin suppression observed in other studies using an equivalent 30 minute mono- or polychromatic light paradigm but not those using longer (90 minute) light exposures. In sum, these data provide evidence for an initial S-cone contribution to melatonin suppression that rapidly decays under extended light exposure.

KEYWORDS

action spectrum, melatonin suppression, S-cone, spectral sensitivity

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day.\textsuperscript{5,6} Given the importance of such “non-visual” actions of light for health, well-being and productivity,\textsuperscript{7,8} there is significant interest in obtaining a detailed understanding of the photoreceptive mechanisms involved that can be used to inform lighting design and practical light therapy interventions.

Over the past 25 years, an overwhelming body of evidence, across human and nonhuman animal models, points to a central role of intrinsically photosensitive retinal ganglion cells (ipRGCs) in driving non-visual responses.\textsuperscript{9,10} The ipRGCs possess their own intrinsic photoreceptive protein, melanopsin,\textsuperscript{11-13} helping to explain initial observations that the spectral sensitivity of circadian and neuroendocrine responses differed from that of perceptual vision\textsuperscript{14-16} and that totally blind patients (lacking rods and cones) could retain such responses.\textsuperscript{17,18} However, in the intact retina, ipRGCs also integrate outer retinal signals originating with rods and cones.\textsuperscript{13,19} In principle, therefore, non-visual responses in humans could involve a combination of signals from any of the five known retinal opsins (melanopsin, rhodopsin, S-, M- and L-cone opsin). Such multi-opsin contributions have been clearly demonstrated for one physiological response under ipRGC control, the human pupil light reflex.\textsuperscript{20-23} A few studies have also provided evidence compatible with multi-opsin contributions to circadian and neuroendocrine responses under certain conditions (eg\textsuperscript{14,24,25}). Understanding the nature and extent of such actions, however, is far less complete than for the pupil response, due in large part to the greater challenges associated with measuring these other effects.

To facilitate comparison of data between laboratories and the generation of models that could predict how specific light exposure patterns might engage non-visual responses, a new measurement framework was proposed that describes effective (α-opic) irradiance for each of the human retinal opsins.\textsuperscript{26} This approach, now formalised as an SI-compliant system of metrology,\textsuperscript{27} has now been widely used to evaluate melanopsin contributions to the spectral sensitivity of various non-visual responses (eg\textsuperscript{28-31}), most notably acute light-induced melatonin suppression which provides the richest pool of data to draw from.\textsuperscript{2,2}

Prior to the widespread appreciation of melanopsin and the ipRGCs, two studies investigated the spectral sensitivity of acute melatonin suppression using various wavelengths of narrowband light (30 or 90 minute exposures).\textsuperscript{14,15} In both cases, the action spectra were reported to exhibit greatest sensitivity in the short wavelength part of the spectrum (~460 nm), significantly different from the visual opsins but also appreciably shorter than would be expected for melanopsin (\(\lambda_{\text{max}}\approx 490 \text{ nm in situ after correction for transmission properties of the human lens}.\))\textsuperscript{26,32} The addition of melatonin suppression data for another wavelength (420 nm)\textsuperscript{33} to the 90 minute exposure action spectrum,\textsuperscript{15} and subsequent re-evaluation of this extended action spectrum revealed the spectral sensitivity did indeed align with that expected for melanopsin alone.\textsuperscript{29,31} By contrast, data from our own (30 minute exposure) action spectrum\textsuperscript{14} could not be adequately explained by the involvement of a single class of opsin, with higher sensitivity to short wavelength light than expected for melanopsin.\textsuperscript{31} Collectively these data have been taken as evidence that S-cones may contribute to early but not later phases of the acute melatonin suppression response. Of note, however, a recent study was unable to detect any differences in the evening rise in melatonin synthesis (measured over the 2.5 hours prior to habitual bedtime) when participants were exposed to lighting conditions that selectively differed in brightness for S-cones.\textsuperscript{34}

Given this uncertainty as to the spectral sensitivity of acute melatonin suppression responses and the potential contribution of S-cones, here we assess irradiance-response relationships for very short wavelength light (415 nm), collected under identical conditions to our original action spectrum.\textsuperscript{14} We then use these new data alongside our previously published data for mono- and polychromatic light exposures\textsuperscript{14,35-37} to validate multi-opsin models that account for the spectral sensitivity of acute light-induced melatonin suppression.

2 | METHODS

2.1 | Participants

Self-reported healthy, drug-free male participants (n = 6) aged 19-34 years (26 ± 6; mean ± SD) completed three study sessions, each of which consisted of three consecutive nights (no light baseline followed by two light exposure sessions) in the Clinical Investigation Unit at the University of Surrey. They all had regular sleep-wake schedules with no complaints of sleep disorders (Pittsburgh Sleep Quality Index ≤5). They had no colour vision deficiencies according to the Ishihara Colour Blindness Test. The study was approved by the University of Surrey Ethics Committee, and all procedures were conducted in accordance with the Declaration of Helsinki. All procedures were carried out with the adequate understanding and written consent of the participants. Other data used for analysis here used comparable participant selection criteria and experimental procedures, as previously described.\textsuperscript{14,35-37}

2.2 | Protocol

This study was conducted between March 2000 and November 2001, shortly following completion of our original action spectra\textsuperscript{14} (May 1998 through to February 2000). The prestudy measurements and protocol were carried out as previously described.\textsuperscript{14} Briefly, each study session (each
spaced a week apart) consisted of three study nights (19:00-07:00 hours): night 1 was a baseline night (no light exposure) followed by two light exposure nights. The three sessions utilised respectively: (a) 25 µW/cm² of 424 nm and 5 µW/cm² of 415 nm, (b) 1 µW/cm² and 26 µW/cm² of 415 nm and (c) 40 µW/cm² and 16 µW/cm² of 415 nm light. The order of stimulus presentation within each session was randomised between participants. Posture and environmental lighting were controlled throughout (21:00-23:00 hours < 10 lux in the direction of gaze; 23:00-07:00 hours 0 lux with participants wearing eye masks except during light exposure). On light exposure nights, the subjects received 30 minutes of 415 or 424 nm monochromatic light. As in our original study, 14 stimulus peak wavelengths and half-maximal bandwidths were based on measured values using a spectroradiometer (Spectrascan 650 portable, Photoresearch). Throughout the study, irradiances were measured with a calibrated radiometer (R203, Macam Photometrics Ltd).

### 2.4 | Data Analysis

As for the original action spectrum, 14 melatonin suppression was calculated as the difference in melatonin concentrations between the corresponding baseline night and the light exposure night averaged across samples collected at 30 and 45 minutes after the start of the 30 minute light exposure (time of maximal melatonin suppression). 39 This facilitated direct comparison with data from our earlier study for monochromatic light where the measured peak wavelengths were 424, 456, 472, 496, 520 and 548 nm (half-maximal bandwidths = 5-13 nm; n = 4-8 participants per intensity/wavelength).

The relationship between log_{10} photons incident at the cornea and melatonin suppression was then determined by fitting data from each wavelength with four parameter sigmoid curves of the form:

\[
Y = \frac{Y_{\text{max}} - Y_{\text{min}}}{1 + 10^{(EC50 - X) \times \text{slope}}} + Y_{\text{min}}
\]

where EC50 is log irradiance producing a response halfway between \(Y_{\text{max}}\) and \(Y_{\text{min}}\) and slope determines the steepness of the curve. We initially constrained data such that \(Y_{\text{max}} = 0\) and tested for differences in EC50, \(Y_{\text{max}}\) and slope between wavelengths via extra-sum-of-squares \(F\) test (GraphPad Prism 7.05, GraphPad Software, Inc.). There were no significant differences in \(Y_{\text{max}}\) \((F_{6,205} = 1.01, P = 0.41)\) or slope \((F_{6,205} = 1.43, P = 0.21)\). Accordingly for all subsequent comparisons, we constrained \(Y_{\text{max}}\) and slope to take the best-fit shared value across wavelengths.

Calculations of equivalent daylight illuminance (EDI) were performed using the relevant \(\alpha\)-opic action spectra and normalisation constants described in the CIE S026 standard 27 in Matlab R2019a (The Mathworks, Inc.). Determination of best-fit single opsins was performed as described previously. 31 In brief, we generated a series of \(\alpha\)-opic templates with arbitrary \(I_{\text{max}}\) based on Govardovskii nomograms 40 and corrected for pre-receptoral filtering, photon energy density and scaling for EDI as described in 27 for melanopic EDI. Multi-opsin models were either derived from details reported in previous publications (“Circadian Stimulus”; CS) 25 or fit to the relevant datasets based on a linear combination of \(\alpha\)-opic EDI and/or photopic illuminance as appropriate. Specifically, we tested models that involved all 5 \(\alpha\)-opic quantities, melanopic, S-cone-opic EDI and photopic illuminance or just simple combinations of melanopic and S-, M- or L-cone-opic EDI. Fits were
optimised in Matlab (using the “lsqcurvefit” function and the 4-parameter sigmoid equation described above), with each of the irradiance quantities making a weighted (positive or negative) influence on the values of $X$. For action spectra comparisons, data for 90-minute exposures from the Brainard group were extracted from the published work$^{15,33}$ using a plot digitiser (WebPlotDigitizer, V 4.1) and an HD Graphics tablet (Wacom Cintiq 16; Wacom Co., Ltd.) as previously reported.$^{31}$

Determinations of the sufficiency of single- or multi-opsin models to explain the data throughout were performed via Extra-Sum-of-Squares F test (GraphPad), with the null hypothesis that EC50s would differ as a function of wavelength. Throughout, goodness of fit for the 4-parameter sigmoid curves that best described the overall datasets (regardless of test wavelength) is reported as adjusted $R^2$ to facilitate comparisons between single- and multi-opsin models with different degrees of freedom. Adjusted $R^2$ was calculated as follows (where $SS_{res}$ and $SS_{tot}$ are the sum of squares of the residuals and overall dataset, $n$ is the number of data points, and $K$ is the number of parameters fit):

$$\text{Adjusted } R^2 = \frac{SS_{res} / (n - K)}{SS_{tot} / (n - 1)}$$

Full details of all the model fits including degrees of freedom, raw and adjusted $R^2$, root mean square error (and equivalent adjusted for differences in fit parameters: $S_{x.y}$) and
corrected Akaike information criteria are provided in Tables S1-S3.

3 | RESULTS

The ability of 415 nm light to acutely suppress plasma melatonin levels was assessed under identical conditions to the previously published action spectrum for nocturnal 30-min light exposures14 (Figure 1A). Analysis of the expanded action spectrum revealed a significant difference in the EC50 but not the slope of the irradiance-response curves (F test; EC50: \( F_{6, 205} = 22.81, P < 0.0001 \); slope: \( F_{6, 205} = 1.43, P = 0.21 \)). Of particular note, sensitivity to 415 nm stimuli was especially low (EC50 = 13.95 photons/cm²/s) such that fivefold more light was required to produce an equivalent response to the most effective wavelength (424 nm, EC50 = 13.19 photons/cm²/s).

We next determined the extent to which the variation in sensitivity across the seven wavelengths in the expanded action spectrum could be explained by a primary contribution of any of the five opsins in the human retina, using the new framework for quantifying ipRGC-influenced responses.27 Consistent with prior analysis of the previously published action spectra,14,31 melatonin suppression measured under these conditions was better described by melanopic EDI than photopic illuminance or any other \( \alpha \)-opic quantity (Figure 1B,C; Figure S1, Table S1). Indeed, the addition of 415 nm data slightly enhanced the degree to which melanopic EDI could explain between-wavelength variations compared to the originally published action spectrum (Figure 1C). Nonetheless, it was equally apparent that melanopic EDI still failed to adequately predict sensitivity to all test wavelengths (Figure 1B; F test; EC50: \( F_{6, 212} = 28.04, P < 0.0001 \)), with the shorter wavelength stimuli (particularly 424 nm) exhibiting lower EC50 than for longer wavelengths.

In line with the inability of melanopic EDI to fully explain the between-wavelength variations in sensitivity, modelling using a series of \( \alpha \)-opic templates (constructed as per the melanopic standard27) revealed the best-fitting single opsin had a \( \lambda_{\text{max}} \) of 467 nm prior to correction for lens transmission (Figure 1D). This value was slightly long wavelength shifted compared to equivalent analysis of the originally published action spectrum14 but still substantially shorter than that expected for melanopsin (480 nm prior to lens transmission correction) and that which best describes the published action spectra for 90-minute light exposures15,29,31,33 (Figure 1D). Most significantly, however, even when we quantified the present data as \( \alpha \)-opic EDI for a single opsin with \( \lambda_{\text{max}} = 467 \) nm, this was insufficient to adequately predict sensitivity across all test wavelengths (Figure 1E,F; F test; EC50: \( F_{6, 212} = 17.34, P < 0.0001 \)).

The findings above therefore imply that, unlike the situation for longer light exposures (where melatonin suppression appears to be dominated by melanopsin contributions15,29,31,33), acute melatonin suppression occurring under the present conditions involves signals from more than one opsin. Accordingly, we went on to evaluate the ability of multi-photoreceptor models to describe the spectral sensitivity of melatonin suppression as measured under the present conditions (Figure 2; Table S2).

The previously proposed CS model,25 based in part on our action spectrum,14 involves a nonlinear combination of signals from rods, cones and melanopsin to provide a single quantity (“Circadian Light”; CLa). Unsurprisingly, CLa very well approximated the relative sensitivities of the original six test wavelengths (adjusted \( R^2 = 0.94 \)); however, it greatly (>1 log unit) over-estimated the sensitivity to the new 415 nm data and therefore failed to adequately describe the full action spectrum reported here (Figure 2A; F test; EC50: \( F_{6, 212} = 25.43, P < 0.0001 \)). An optimised linear combination of the \( \alpha \)-opic EDIs for the five retinal opsins, which provided both a nominally better fit to the original action spectrum14 (adjusted \( R^2 = 0.95 \)), came closer to predicting the 415 nm data (−0.67 log units) but still failed to adequately describe the full action spectrum (Figure 2A; F test; EC50: \( F_{6, 212} = 12.76, P < 0.0001 \)).

We next then evaluated simpler models involving signals from just melanopsin and/or one or more classes of cone opsins. Models based on just melanopic and M- or L-cone-opic EDI failed to account for the difference in sensitivity even across the six wavelengths in our original action spectrum14 (Figure S2A,B). By contrast, models comprising a linear weighting of melanopic and S-cone-opic EDI, with (Figure 2C) or without (Figure 2D) the inclusion of photopic illuminance, provided a substantially better description of the data overall, even relative to the more complex models like CS that involve all opsin classes. Indeed, the simplest model fit to the original six test wavelengths (Figure 2D, a weighted combination of 54.1% melanopic and 45.9% S-cone-opic EDI) provided the closest prediction of the 415 nm data (−0.4 log units) and the best overall fit to the extended action spectrum (adjusted \( R^2 = 0.87 \)). Indeed, the overall prediction error for this model (root mean square error across all test stimuli) was significantly lower than for a melanopsin only model (Figure S2B; Friedman test with Dunn’s multiple comparison test, \( P < 0.001 \)). Importantly, however, even this best-fit melanopsin/S-cone model was still unable to fully account for the variations in EC50 across test stimuli in the full action spectrum (Figure 2D; F test; EC50: \( F_{6, 212} = 11.97, P < 0.0001 \)).

The inclusion of a substantial melanopic component in the melanopsin/S-cone model, as well as the melanopsin/cones model (which was nearly identical barring the addition of a modest photopic illuminance component), is in keeping with
decades of research highlighting a central role for ipRGCs in nonvisual responses. Nonetheless, since none of the models evaluated above fully accounted for the variations in EC50 across test stimuli, we also determined the extent to which models based solely around cone-opic illuminance might be able to better approximate the apparent spectral sensitivity of melatonin suppression. One such model, comprising positive S- and M-cone contributions and negative L-cone contributions, performed almost as well as the melanopsin/S-cone model (“All cones”; Figure S2, Table S2). Nonetheless, like other more complex models we evaluated, this All cones model was unable to account for significantly more of the variance in the data than the more simple melanopsin/S-cone model (Figure S2B) which, by virtue of the inclusion of a melanopsin component, best aligns with the expected biology.

Since none of the multi-opsin models based on the original action spectrum could fully explain the variation in melatonin suppression across test stimuli, we next repeated the model fits including all seven wavelengths making up the expanded action spectrum (Figure 3A-C; Figure S2C; Table S3). While, as expected, these models produced fits with nominally higher adjusted $R^2$, none of these models could fully account for the variations in EC50 across test stimuli (Figure 3A-C; $F$ tests, all $P < 0.0001$) or resulted in significantly lower prediction errors than the best-fitting melanopsin/S-cone model (Figure S2D; Friedman test with Dunn’s multiple comparison test, all $P > 0.05$). As noted above, however, some of the other models did provide a nearly equal approximation of apparent spectral sensitivity; either because the cone contribution was minimal (“Melanopsin+cones” model) or by virtue of the inclusion of

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**FIGURE 2**  Sensitivity of melatonin suppression to short wavelength light is best explained by a combination of S-cone and melanopsin signals. Upper Panels: show data from quantified using a nonlinear multi-opsin model (A; “Circadian Light”, CLa) or via best-fit linearly weighted combinations of a-opic EDI from all 5 opsins (B), melanopic, S-cone-opic and photopic EDI (C) or only melanopic and S-cone-opic EDI (D). Adjusted $R^2$ values reflect variance explained by best-fit 4-parameter sigmoid curve across all wavelengths (black lines), accounting for differences in degrees of freedom across models incorporating different combinations of opsins. $P$ values indicate extra-sum-of-squares $F$ test for null hypothesis that EC50s will not differ. Middle Panels: Comparison of measured response to 415 nm stimuli vs that predicted from model fits in corresponding upper panels. $\Delta$ sens. indicates the log difference in sensitivity between measured and predicted EC50; $P$ value indicates extra-sum-of-squares $F$ test for null hypothesis that EC50s will not differ from predicted value. Lower panels: show best cross-wavelength 4-parameter sigmoid curves (dotted black lines) for the full action spectrum, including 415 nm data. Conventions as in upper panels.
negative L-cone weights (“All opsins” and “All cones” models). In the case of the latter, this negative weighting helped to mimic the long wavelength limb of the melanopsin spectral sensitivity curve across the wavelength range tested here (Figure S3). Notably, however, it also meant that spectral sensitivity went on to fall precipitously, becoming undefined (ie negative) wavelengths longer than those contributing to the present dataset. Such models are most unlikely therefore to provide an accurate description of the underlying biology and would not be applicable to other datasets using light enriched in longer wavelengths (eg15,35).

On aggregate, the implications of our analysis are that the spectral sensitivity of melatonin suppression under the present conditions is best approximated by a combination of S-cone and melanopsin signals and that considering contributions from other opsins does not improve this. Since even tailor-fit, five opsin, models could not fully describe the data, we conclude that residual variations in EC50 with the melanopsin/S-cone model (Figure 3C) more likely originate with differences in light sensitivity across cohorts of participants contributing data for each wavelength. To assess the extent to which such variance might influence the apparent contribution of melanopic vs S-cone-opic EDI, we calculated the combination of those two signals that best described the relative sensitivity of melatonin suppression to subsets of irradiance-response relationships comprising five of the seven wavelengths making up the action spectrum (Figure 3D). All the test wavelength combinations included a positive S-cone contribution (Wilcoxon signed-rank test vs expected value of 0, \(P = 0.002\)). Moreover in all cases, the corresponding model fits applied to the full new dataset resulted in a substantially higher adjusted \(R^2\) than for a melanopsin only model (Figure 3D; adjusted \(R^2 = 0.82-0.89\), Wilcoxon signed-rank test vs melanopsin only value of 0.75, \(P = 0.002\)). We did, however, note that combinations of test wavelengths that included 415 nm but not 424 nm
irradiance-response relationships resulted in lower estimates of S-cone contribution than the converse (415 nm: 16.7 and 21.4% vs 424 nm: 47.7%-49.9% S-cone). By contrast, combinations of wavelengths that included both 415 and 424 nm or neither of those two wavelengths provided intermediate estimates (31.3%-41.8% and 33.8% S-cone) which aligned with that obtained from a fit to the full new dataset (Figure 3C; 34.5%).

A parsimonious interpretation of the data above is that some baseline difference in light sensitivity between cohorts of participants contributing to 415 and 424 nm explains the residual variation in sensitivity when light exposure is quantified according to the best-fit melanopsin/S-cone model (Figure 3C; Figure 4A). Any such variation could not simply be ascribed to subject age; the ages of participants contributing to the 415 nm data (19-34; mean = 26 years) were similar to those contributing to the original action spectrum (18-45; mean = 27 years). 14 Moreover, inter-individual differences in sensitivity to 415 nm light in the present study displayed no overt relationship to participant age (Figure S4A,B). Nonetheless, evaluation of responses to a probe 424 nm exposure (25 µW/cm²; 13.9 log photons/cm²/s) among the participants that contributed to the 415 nm dataset did reveal evidence consistent with a difference in baseline light sensitivity. Hence, melatonin suppression produced by the probe stimulus fell just below the 95% confidence intervals of a 4-parameter sigmoid curve fit to the original 424 nm irradiance-response relationship14 (Figure S4C). Importantly, however, melatonin suppression evoked by the probe 424 nm stimulus almost perfectly matched that predicted by the overall S-cone/melanopsin model fit to the extended action spectra (Figure S4D). This finding therefore provides confidence that modelling based on the full new dataset is not unduly influenced by the possibility of differences in baseline light sensitivity between cohorts of subjects tested across the various wavelengths.

To provide additional confidence that a melanopsin/S-cone model recreates the spectral sensitivity of acute melatonin suppression occurring under the conditions evaluated here, we further examined data from independent studies that used an equivalent paradigm. Specifically, we evaluated three studies15-37 that compared melatonin suppression evoked by 30-min exposure to monochromatic and various polychromatic mixtures, in participants with dilated pupils. As expected, the melanopsin/S-cone model (from Figure 3C) resulted in a nominally better fit to the aggregated data, compared to a melanopsin only model (Figure 3E,F; adjusted $R^2 = 0.65$ vs 0.64). By contrast, action spectrum data for longer (90 minutes) light exposures,15,33 where previous analysis suggested an effectively pure melanopsin contribution,29,31 were less well fit by the dual opsin model (Figure 4B,C; adjusted $R^2 = 0.85$ vs 0.88 for melanopsin only). In sum, these data are therefore consistent with a model whereby S-cones noticeably contribute to melatonin suppression during early but not later stages of light exposure.

4 | DISCUSSION

The present data provide clear evidence for an S-cone contribution to acute suppression of pineal melatonin production in humans. Analysis of melatonin suppression evoked by longer (90 minutes+) light exposures indicates the spectral sensitivity of such responses is almost exclusively determined...
by melanopsin.\textsuperscript{15,29,31,33,41} By contrast, while the present data certainly suggest a strong melanopsin contribution to melatonin suppression evoked by 30-minute exposures, the sensitivity to wavelengths shorter than the melanopic peak is consistently higher than expected relative to longer wavelengths. Indeed, based on our modelling, a parsimonious interpretation of the available data indicates a combination of just melanopsin and S-cone-opic irradiance (at an \(\sim 2:1\) ratio) dictates the extent to which light exposure will suppress melatonin under the present conditions. Based on the absence of an equivalent short wavelength bias in the spectral sensitivity of melatonin suppression following longer light exposures, we further conclude that S-cone contributions to melatonin suppression decay rapidly upon extended light exposure.

The idea that cone signals dominate initial components of ipRGC-dependent responses while later components become increasingly melanopsin dominated is well established for the pupal system.\textsuperscript{20-23} In that case, however, the inferred properties of human ipRGCs (based both on the later pupal data and physiological recordings from macaque ipRGCs that project to the pretectum\textsuperscript{15}) is that they convey S-[L+M] (blue-yellow) cone-opponent signals in addition to those originating with melanopsin. Such properties diverge, therefore, from the additive combination of melanopsin and S-cone signals that account for melatonin suppression under the present conditions. It is noteworthy that there are multiple subtypes of ipRGCs in humans which are likely to have distinct functional properties and/or projection targets.\textsuperscript{42,43} It is possible, therefore, that the ipRGCs responsible for the melatonin suppression response receive different sets of cone inputs from those regulating the pupil and/or that more complex network mechanisms downstream of ipRGCS are involved in shaping the apparent spectral sensitivity.

In accordance with the idea of comparatively transient cone influences on ipRGC-influenced responses of more relevance to the present study, a previous investigation of the time course of melatonin suppression evoked by long 6.5 hour exposures to short and long wavelength narrowband light has provided evidence for a rapidly decaying L-/M-cone contribution.\textsuperscript{24} That study did not include the shorter wavelengths needed to reliably evaluate S-cone contributions, but did find evidence that a contribution of L- and/or M-cones (photopic illuminance) influence initial phases of melatonin suppression, with sensitivity to 460 and 555 nm being virtually identical during the first 30-min light exposure. By contrast, in the present study we did not find clear evidence for any L-/M-cone contribution, with an equivalent pair of wavelengths (456 vs 548 nm) differing by \(-0.9\) log units in sensitivity. Moreover, for multi-opsin models that included photopic illuminance, the weighting of this component was negligible (<5%) and did not significantly improve fits to the data compared to more simple melanopsin/S-cone models. While the origin of this difference in the spectral sensitivity of acute melatonin suppression will await definitive confirmation, this may reflect the very different experimental protocols between the present study and that of Gooley et al.\textsuperscript{24} Indeed, in the latter study participants were housed under very low light levels (<3 lux) for several days prior to the experimental light exposure, which may conceivably have sensitised L-/M-cone contributions.

Another previously proposed multi-opsin model (CS), based in part on data from our original action spectrum,\textsuperscript{14} includes a nonlinear combination of photoreceptor signals including a blue-yellow colour-opponent mechanism.\textsuperscript{25} Importantly, that model greatly overestimates sensitivity to the new 415 nm data reported here and we find no clear evidence to support a blue-yellow colour-opponent influence on melatonin suppression. Instead, as discussed above, our data suggest L-/M-cone influences on melatonin suppression are negligible under the present conditions. We should, however, note that none of the models we evaluated were able to fully account for the variation in sensitivities across all test wavelengths. Since even the most complex models evaluated could not outperform a simple melanopsin/S-cone model, we infer the residual variance in sensitivity observed with that model \((-0.3\) log unit difference in EC50s) originates with technical factors, rather than any meaningful difference in effective spectral sensitivity from that we modelled. Importantly, the procedures we used to collect and analyse the 415 nm data for the present study were identical to the original action spectrum (and participants were well matched in age; mean age of 26 years in the present study vs 27 years in\textsuperscript{14}), ruling out obvious methodological differences. Indeed, we specifically confirmed that variations in participant age across the range studied did not significantly influence sensitivity to the 415 nm stimuli used here (Figure S4A,B). The recent identification of significant \((-40\)-fold) inter-individual variability in the light sensitivity of melatonin suppression (of unknown origin)\textsuperscript{44} does, however, suggest a generalised between-cohort variation in light sensitivity could underlie the residual variance in our melanopsin/S-cone model. Indeed, our analysis of responses to a probe 424 nm stimulus in participants contributing to the new 415 nm irradiance-response curve described here supports the view that between-cohort differences contribute to the residual variation in that model.

As for the individual irradiance-response relationships in another previous action spectrum,\textsuperscript{15,33} the new data reported in this study were collected using a within-participants design (each of which received all irradiances). By contrast, in our original action spectrum,\textsuperscript{14} participants were randomly assigned across wavelengths/irradiances, specifically to mitigate the possibility that between-cohort variation might skew the overall assessment of spectral sensitivity. Nonetheless, given the wide (and almost bimodal) distribution of light sensitivity for melatonin suppression reported recently,\textsuperscript{44} it is entirely feasible that even randomly selecting cohorts of
participants for each irradiance could result in irradiance-
response curves that deviated sufficiently from the “true” pop-
ulation mean sensitivity to account for the residual variance in
our model. To control for the possibility that variations of this
nature are sufficiently large as to provide a misleading picture
of the overall spectral sensitivity, we perform model fits on
subsets of the irradiance-response relationships contributing
to the full action spectrum. This analysis consistently reveals
a positive S-cone contribution to spectral sensitivity of acute
melatonin suppression. The relative contribution of S-cones
is estimated as higher for models that include the 424 nm and
not 415 nm data and lower for those that lack 424 nm but
contain 415 nm data, suggesting that the apparent sensitivity
to one or both of those wavelengths deviates from the “true”
population sensitivity. Our further analysis suggests that in
fact the 424 nm data reflect a modest over-estimation of sen-
sitivity and the 415 nm data a modest underestimate and that
this opposing variability cancels out in the overall model fits.
Hence, model fits that include both or neither of those wave-
lengts consistently result in models with very similar (inter-
mediate) melanopsin/S-cone weighting.

A previous report that S-cones do not contribute to acute
neuroendocrine responses to light34 is, therefore, seemingly
at odds with the present conclusions. That study used lighting
conditions that selectively differed in S-cone-opic irradiance
(almost 100-fold) and found no difference in the evening rise
in melatonin (measured 2.5-0.5 hours prior to habitual bed
time). An important methodological difference between that
study and the present work is that here data were collected in
participants with pharmacologically dilated pupils, whereas
mydriatics were not used by Spitschan et al.34 Cone influences
on the pupil response could indirectly influence the spectral
sensitivity of melatonin suppression responses (by changing
retinal exposure levels). It should be noted, however, that, if
anything, the reported S-cone-opponent contribution to pupil
control21,22 might be expected to enhance rather than reduce
a more direct contribution of S-cone pathways to control mel-
onin production. Perhaps more likely, then, the lack of an
ovet S-cone contribution in34 reflects the fact that the light
stimuli employed exhibit less S-cone vs melanopsin bias than
the narrowband stimuli used in the present study (Table S4).
Certainly, inspection of the data in34 indicates a tendency to-
wards reduced melanin synthesis during early phases of the
high S-cone stimulus, which is broadly in keeping with our
model predictions of an ~2:1 melanopic:S-cone-opic irradi-
ance contribution to early phases of light-induced melatonin
suppression.

A final point to consider, when interpreting the data pre-
9ented here, is that we did identify additional multi-opsin
models that provided almost equivalent approximations of the
apparent spectral sensitivity of melatonin suppression to the
Melanopsin/S-cone model. Critically, all such models
(“All opsin”, “Melanopsin/Cones” and “All cones”) included
a substantial positive S-cone contribution. Indeed, there is
no biologically plausible manner in which the high short
wavelength sensitivity observed here could arise without the
inclusion of S-cones. By contrast, while the integrated spec-
tral sensitivity of all these models (as illustrated in Figure
S3) is similar to that of the Melanopsin/S-cone model for the
range of wavelengths tested in the present study, the photo-
receptive origin of sensitivity to longer wavelengths varies
substantially. Hence, while sensitivity of the long wavelength
limb is primarily determined by the melanopic curve in the
Melanopsin/S-cone and Melanopsin/Cones models, in other
models it involves (“All opsins”), or is entirely generated by
(“All cones”), a combination of positive M-cone and negative
L-cone contributions. Since the inclusion of negative L-cone
contributions in these latter models renders the overall spec-
tral sensitivity for wavelengths >555 nm undefined, we do
not consider these biologically plausible models. Conversely,
a melanopsin component to melatonin suppression is entirely
in keeping with the well-established understanding of the un-
derpinning biological mechanisms.11-24 Nonetheless, these
data certainly highlight the possibility that, in principle, other
opsins beyond melanopsin and S-cones could contribute to
melatonin suppression under the conditions studied here, pro-
vided they are combined in a manner that does not change
sensitivity to longer wavelengths substantially from that ac-
counted for by melanopsin alone.

In summary, the present data provide new insight into
the photoreceptor mechanisms regulating human non-visual
responses, by defining an S-cone contribution to the initial
cute suppression of pineal melatonin production. These
data therefore support previous suggestions that avoiding
short wavelength enriched light during the evening/night
may benefit sleep and circadian health. Given previous data
suggesting that subjective alertness following early morning
light exposure is also especially sensitive to very short wave-
lengts,45 potential S-cone contributions to other acute non-
visual responses is an important area for further investigation.

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CONFLICT OF INTEREST

KT, JA and DJS are co-inventors on an issued patent (EP1614444A1), and VLR and DJS are co-inventors on an issued patent (WO2015052207A1). VLR is a scientific advisor to Lumie.

AUTHOR CONTRIBUTIONS

KT, JA, VLR and DJS designed the studies. KT and VLR collected the data. KT, VLR, DJS and TMB analysed the data and wrote the manuscript.

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 may be found online in the Supporting Information section.

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