INTRODUCTION

Light exerts a range of physiological responses in humans, including synchronising the circadian system and regulation of melatonin secretion, sleep/alertness and pupil constriction.1-4 Given the potential importance of such “nonvisual” responses for health and well-being, there is significant interest in determining the nature of the sensory signals responsible and in establishing appropriate ways to quantify and predict such effects.

It is now clear that nonvisual responses primarily originate via intrinsically photosensitive retinal ganglion cells (ipRGCs) which combine intrinsic, melanopsin-based, phototransduction with extrinsic rod/cone-mediated signals. As a result of this arrangement, it has remained unclear how best to quantify light to predict its nonvisual effects. To address this, we analysed data from nineteen different laboratory studies that measured melatonin suppression, circadian phase resetting and/or alerting responses in humans to a wide array of stimulus types, intensities and durations with or without pupil dilation. Using newly established SI-compliant metrics to quantify ipRGC-influenced responses to light, we show that melanopic illuminance consistently provides the best available predictor for responses of the human circadian system. In almost all cases, melanopic illuminance is able to fully account for differences in sensitivity to stimuli of varying spectral composition, acting to drive responses that track variations in illumination characteristic of those encountered over civil twilight (~1-1000 lux melanopic equivalent daylight illuminance). Collectively, our data demonstrate widespread utility of melanopic illuminance as a metric for predicting the circadian impact of environmental illumination. These data therefore provide strong support for the use of melanopic illuminance as the basis for guidelines that seek to regulate light exposure to benefit human health and to inform future lighting design.

KEYWORDS

circadian, colour, irradiance, light, melanopsin, melatonin, retina
(ipRGCs) whose axons innervate the hypothalamus and other subcortical regions.\textsuperscript{5-7} The ipRGCs’ intrinsic photopigment, melanopsin, explains previous observations that nonvisual responses persist in the functionally blind.\textsuperscript{8-13} However, in the visually intact, ipRGCs also receive extrinsic signals originating via rods and cones.\textsuperscript{14} As such, nonvisual responses can, in principle, originate via a combination of any of the five retinal opsins (melanopsin, rhodopsin, S-, M- and L-cone opsins). Moreover, existing data suggest that the relative contribution of these various signals is likely to vary depending on the irradiance and duration of light exposure.\textsuperscript{2,9,15-17}

As an initial response to the challenges associated with quantifying light according to its nonvisual effects, Lucas et al\textsuperscript{2} proposed a system of α-opic metrics, where illuminance was quantified according to its effective impact for each of the five known human opsins. This approach has now been formalised, with minor modifications, as an SI-compliant system of metrology, whereby the α-opic values are equal to photopic illuminance for natural daylight (equivalent daylight illuminance; EDI).\textsuperscript{18}

While this accepted scientific framework now makes it possible to incorporate nonvisual impact as a design consideration for lighting and visual displays, applications of this nature are hampered by uncertainty regarding how each of the five opsins contribute. Recent reports highlight melanopic illumination as a key factor in at least some circumstances.\textsuperscript{19-21} The extent to which this varies depending on the specific conditions or nonvisual response being studied is largely unclear, however. Here then, we address this uncertainty by reanalysing data from a large number of studies that evaluated the spectral sensitivity of melatonin suppression,\textsuperscript{22-35} circadian phase resetting\textsuperscript{29,34-36} and/or alerting responses,\textsuperscript{32,33,37} encompassing a wide variety of stimulus durations and types and including data from subjects with dilated or freely responsive pupils.

2 | MATERIALS AND METHODS

2.1 | Study selection criteria

In order to minimise methodological differences as a source of variability in our analysis, rather than attempt an exhaustive meta-analysis, here, we set out to specifically identify those laboratory studies where it was possible to perform meaningful within-study assessments of spectral sensitivity. In particular, our primary inclusion criteria were those studies that analysed melatonin suppression and/or circadian phase resetting in response to at least 2 spectrally distinct sources (of identified spectral composition) delivered at multiple intensities, together providing at least 6 different stimuli. We further limited our analysis to data collected in young to middle aged adult subjects (mean ages of experimental groups 20-45) to best match the standard observer spectral sensitivity upon which the CIE standards are based\textsuperscript{18} and to studies evaluating light exposure during the evening/early night (ie times when light exerts a phase-delaying effect on the circadian system). Care was taken not to include any data more than once (in those cases were preliminary data sets were subsequently incorporated as part of more extensive later studies).

For some of the more practically relevant conditions, we hoped to evaluate (circadian phase resetting and melatonin suppression following long light exposures in subjects with undilated pupils) there were no studies that met the criteria above. In those specific cases, then, we relaxed the requirement for testing multiple light intensities and aggregated data across studies, provided the stimuli were administered in a comparable manner (ie they tested a single nocturnal light exposure per experimental session) and that the aggregated data set met all our requirements above. Among the identified studies, a number also evaluated alerting responses by means of the Karolinska sleepiness scale (KSS). Although not our primary aim, we therefore undertook here a preliminary evaluation of such data where results could be meaningfully aggregated across studies.

In total, we identified 14 studies on the spectral sensitivity of melatonin suppression\textsuperscript{22-35} and 4 studies of circadian phase resetting\textsuperscript{29,34-36} that met our criteria. These all employed single continuous light exposures during the evening/night in adult subjects but differed in the use of pupil dilators and the nature and duration of light stimuli (as outlined below and summarised in Table 1 and Table S1). Our preliminary analysis of the spectral sensitivity of alerting responses was based on 3 studies that reported raw KSS values during light exposures in subjects with dilated pupils across comparable portions of the late evening.\textsuperscript{32,33,37} For validation of irradiance-response relationships derived from analyses which aggregated data across several studies, we further analyse data from 3 studies that did not investigate spectral sensitivity but provide useful comparator data (ie irradiance-response relationships for white light-driven melatonin suppression, circadian phase resetting or alerting responses).\textsuperscript{38-40}

2.2 | Study details

Four studies\textsuperscript{22-25} compared melatonin suppression to full-field 30 minutes light exposures, administered to subjects with dilated pupils during the evening melatonin rise (23:30 to 03:00 hours). Light-evoked changes in plasma melatonin were expressed as percent relative to a control night\textsuperscript{22} or to prelight exposure.\textsuperscript{23-25} Stimuli employed were narrowband light at wavelengths between 424 and 548 nm\textsuperscript{22} or comparisons between narrowband 479 nm light and mercury vapour
lamps, warm and cool fluorescent sources and various dichromatic narrowband mixtures.

The Brainard group reported action spectra for melatonin suppression evoked by full-field 90 minutes exposures to narrowband sources (420-600 nm) between 02:00 and 03:30 hours in subjects with dilated pupils. Melatonin suppression was analysed as percent change between samples taken at the end-start of the protocol, normalised to no-light control exposures. Najjar et al also used a near-identical paradigm to compare melatonin suppression to narrowband sources (420-630 nm) of equal photon flux. Further, the Brainard group used equivalent approaches to evaluate responses to 90 minutes polychromatic fluorescent light exposures in subjects with undilated pupils.

Additional evaluations of melatonin suppression in subjects with undilated pupils were provided by several studies. Nagare and colleagues used 3 hours exposures to warm and cool white LED-based stimuli via desktop light boxes between 23:00 and 02:00 hours, with control-adjusted percent suppression reported for each hour of the stimulus (note only data from adults subjects were included in this analysis). Rahman et al measured melatonin suppression (control adjusted AUC) produced by 8 hours exposures (16:00 to 24:00) to broadband fluorescent and LED sources via wall-mounted light boxes. Santhi and colleagues measured melatonin profiles from subjects exposed to 4 hours warm or cool fluorescent light before habitual bedtime via wall-mounted light boxes (for analysis here, reported melatonin AUCs were expressed as a percentage of the mean near-dark control values). These latter two studies also report raw KSS values at regular intervals during the light exposure paradigm. Similarly, Chellappa et al report equivalent KSS data for subjects receiving a 2 hours evening exposure to warm or cool fluorescent and incandescent sources (indirect exposure in a spectrally neutral room). For preliminary analysis of alerting responses, we used KSS data obtained 70-90 minutes prescheduled/habitual sleep times (at which point the subjects had been exposed to the experimental sources for > 1 hour).

Gooley et al measured melatonin suppression and circadian phase resetting following 6.5 hours exposures to full-field monochromatic light (460 vs 555 nm), centred around the acrophase of the melatonin rhythm, in subjects with dilated pupils. Melatonin suppression was calculated as area under the curve (AUC), adjusted according to values for dim light control from the preceding cycle and reported for each quartile of the stimulus. Phase shifts were calculated as the change in dim light melatonin onset (DLMOn) between the day after and before light exposure. Other studies also used essentially identical

| Table 1 | Summary of studies analysed. Provides details on end points and test stimuli for all the studies used in this analysis |
|---|---|---|---|---|---|---|---|
| Ref. | Citation | Pupils | Assay | Stimulus | Spectra | Irrad. | n/group | Dur (min) |
| 22 | Thapan et al 2001 | D | MS | N | 6 | 4-8 | 5-7 | 30 |
| 23 | Revell and Skene 2007 | D | MS | P | 2 | 3 | 9 | 30 |
| 24 | Revell et al 2010 | D | MS | N/P | 7 | 1-2 | 12 | 30 |
| 25 | Papamichael et al 2012 | D | MS | N/P | 7 | 1-4 | 10 | 30 |
| 26 | Brainard et al 2001 | D | MS | N | 8 | 6-10 | 8 | 90 |
| 27 | Brainard et al 2008 | D | MS | N | 1 | 8 | 8 | 90 |
| 28 | Najjar et al 2014 | D | MS | N | 9 | 1 | 5 | 90 |
| 29 | Gooley et al 2010 | D | MS/PS | N | 2 | 24 | 1 | 390 |
| 30 | Nagare et al 2019 | UD | MS | P | 2 | 4 | 8 | 180 |
| 31 | Brainard et al 2015 | UD | MS | P | 3 | 9 | 8 | 90 |
| 32 | Rahman et al 2017 | UD | A/MS | P | 2 | 1 | 16 | 480 |
| 33 | Santhi et al 2010 | UD | A/MS | P | 3 | 1-2 | 19-22 | 240 |
| 34 | Ho Mien et al 2014 | UD | MS/PS | N/P | 2 | 1 | 6-7 | 360 |
| 35 | Hanifin et al 2019 | UD | MS/PS | P | 2 | 1 | 10 | 390 |
| 36 | Wright and Lack 2001 | UD | PS | N | 5 | 1 | 15 | 120 |
| 37 | Chellapa et al 2014 | UD | A | P | 3 | 1 | 16 | 120 |
| 38 | Phillips et al 2019 | UD | MS | P | 1 | 7 | 22-33 | 240 |
| 39 | Zeitzer et al 2000 | UD | PS | P | 1 | 21 | 1 | 390 |
| 40 | Cajochen et al 2000 | UD | A | P | 1 | 20 | 1 | 390 |

Note: D/UD represents dilated and undilated pupils, respectively. A, MS and PS indicate that the study reported alertness, melatonin suppression and/or circadian phase shift data that were used in the present analysis. Note that ref.27 tests only one spectra but is an extension of the more extensive data set presented in ref.26. See also Table S1 for additional study information and details of data corrections applied.
experimental protocols to assess melatonin suppression and circadian phase resetting in subjects with undilated pupils: Ho Mien et al. employed full-field 6 hours exposures to full-field narrowband (631 nm) and fluorescent white light (data for intermittent light exposure in that study were not included in the present analysis); Hanifin and colleagues provided 6.5 hours exposures to warm and cool fluorescent sources presented via large wall-mounted panels. Wright and Lack evaluated circadian phase delays evoked by 2 hours exposures to narrowband (470-660 nm) LED light (00:00 to 02:00 hours) by comparing DLMOn between test day and the day following light exposure.

Analysis derived from some of the above data sets were compared against relevant published white light irradiance-response relationships determined in subjects with undilated pupils. Phillips and colleagues monitored melatonin suppression in response to white light exposure over 4 hours prior to habitual bedtime (calculated as AUC during light exposure relative to a control night). Zeitzer et al. and Cajochen et al., respectively, measured irradiance-response relationships for circadian phase delays and alertness (KSS) following 6.5 hours light exposures centred around the acrophase of the melatonin rhythms as for most other circadian studies above.

2.3 Analysis procedures

Data for responses (mean ± SEM or for individual subjects as appropriate) and, where relevant, corresponding stimulus intensities were extracted from the highest resolution figures available from the published papers using a plot digitiser (WebPlotDigitizer, V 4.1) and an HD Graphics tablet (Wacom Cintiq 16; Wacom Co., Ltd.). Where relevant information was presented in tabular form (or in the manuscript text), those values were used preferentially but in all cases there was a very strong correspondence with measured values. For studies of circadian phase resetting, where necessary, data were adjusted by subtracting reported shifts under dim light conditions or the expected phase drift for that protocol (0.2 h/d) if dark controls were not reported as specified in Table S1.

For studies using polychromatic sources, stimulus spectral compositions were digitised as above. In a few cases where these details were lacking, spectra were estimated based on fluorescent illuminants with equivalent colour temperatures from another publication. For narrowband sources, spectra were modelled as Gaussians with full width at half maximum (FWHM) equivalent to those reported in the original publications. In all cases, resulting spectra were scaled based on intensity information provided in the publications (see Table S1) and converted to α-opic EDI as specified previously using Matlab R2019a (The Mathworks, Inc). Throughout the text, references to α-opic illuminance (eg melanopic illuminance) reflect these EDI values (ie using a scaling procedure based on the D65 daylight illuminant) rather than previous variants that used a different (equi-energy) scaling. For unbiased assessments of best-fit opsin templates (Figure S1B and Figure 2C), we generated a series of α-opic templates with arbitrary λmax based on Govardovskii nomograms corrected for prereceptoral filtering, photon energy density and scaling for EDI as for the melanopic template.

For subsequent assessment of spectral sensitivities, we determined the best-fitting four-parameter sigmoid (GraphPad Software, Inc), weighted to account for mean and scatter in the data and constrained to have a minima at 0. For melatonin suppression data, fits were further constrained to have a maxima ≤100%. Where relevant, R² values reported in the text reflect the correspondence between the fitted curves and the mean responses across the relevant data sets (for more direct comparison with values reported elsewhere in the literature eg). When possible (analysis of individual studies that measured irradiance-response relationships for at least two spectrally distinct stimuli), we further determined whether the fitted curve was sufficient to account for the variability in sensitivity across stimulus types by extra sum of squares test (implanted in GraphPad and referred to in text simply as F test for brevity). Specifically, this test asks whether individual curve fits to data (expressed as α-opic illuminance vs response) for each distinct spectra account for significantly more of the corresponding variance that does a global fit to all data points (irrespective of spectra). A significant result therefore indicates that spectral sensitivity is not reliably predicted by the α-opic quantity in question.

3 RESULTS AND DISCUSSION

3.1 Melatonin suppression in subjects with dilated pupils

We first examined the action spectra for melatonin suppression to brief (30 minutes) nocturnal light exposure in
subjects with dilated pupils. This preceded identification of ipRGCs and reported a λ\text{max} (459 nm) which diverged from that of any known human opsin (including melanopsin; ~480 nm\textsuperscript{7,15,44}). On conversion of those data to α-opic illuminances, we found that sensitivity across wavelengths was most similar when quantified as melanopic (Figure 1A) rather than photopic illuminance, or any of the other α-opic quantities (Figure 1A,B; Figure S1A). However, when quantified as melanopic illuminance, there was still a clear divergence in the irradiance-response curves, with an apparent increase in sensitivity for very short wavelength stimuli (424-440 nm). As such, statistical analysis indicated a low probability that the data could be fully accounted for by melanopsin alone (Figure 1A; \(F\) test: \(F_{15, 157} = 11.5, P < .0001\)).

Consistent with the original report, subsequent modelling based on α-opic templates with arbitrary peak sensitivity (see methods) identified that the best-fitting single opsin had a λ\text{max} of 463 nm (Figure S1B). Importantly, however, even with using this best-fit opsin template, there was a low probability that all the data points could be described by a common function (Figure S1C; \(F\) test: \(F_{15, 157} = 7.1, P < .0001\)). This suggests, therefore, that at least two different opsins may contribute to melatonin suppression under the conditions tested. Indeed, the apparent peak sensitivity falls almost perfectly between the melanopic and s-cone opic quantum efficiency curves (Figure S1B) suggesting a contribution from both of those opsin classes.

To further evaluate the possibility that opsins other than melanopsin make important contributions to melatonin suppression responses under the conditions studied by Thapan et al., we next evaluated studies from the same group that compared near-peak melanopic efficiency monochromatic light (479 nm) against various polychromatic sources.\textsuperscript{23-25} The individual studies reported increased or decreased efficiency of broadband “white” light and unchanged efficiency for polychromatic narrowband mixtures against melanopsin-matched monochromatic light. Aggregating data across all those studies, we found the degree of melatonin suppression could again be better predicted by melanopic rather than photopic or any other α-opic illuminance (Figure 1C,D).

In sum, we conclude that even for relatively brief light exposures in subjects with dilated pupils, melanopic

![FIGURE 1](image)

**FIGURE 1** Melanopsin and cone-weighted illuminance-response relationships for melatonin suppression following brief nocturnal light exposures in subjects with dilated pupils. A, Data from showing mean ± SEM melatonin suppression evoked by 30 min monochromatic light pulses, quantified as melanopic, photopic or S-cone-opic equivalent daylight illuminance (EDI). Dashed curves show global best-fit 4-parameter sigmoid regardless of test wavelength. B, Goodness of fit \(r^2\) for the corresponding global 4-parameter sigmoid curve fits to data expressed as melanopic, photopic and other α-opic illuminances. \(F\) test comparisons indicated a low probability (<.0001) that the global fits could account for variation in sensitivity across wavelengths in all cases. C, Data from that compared melatonin suppression (mean ± SEM) evoked by 30 min monochromatic and polychromatic sources. D, \(r^2\) for the corresponding global 4-parameter sigmoid curve fits.
illuminance provides the best available metric to predict melatonin suppression. Further, while the original action spectra provide evidence that S-cones can also contribute to melatonin suppression under these conditions, equivalent data for polychromatic stimuli do not reveal any systematic increase in sensitivity for stimuli providing comparatively high S-cone stimulation. As discussed below, a recent study in subjects with freely responsive pupils that specifically investigated possible S-cone contributions to melatonin suppression also failed to find evidence of any such effect.45 Accordingly, it seems any contribution from S-cones is unlikely to significantly compromise the predictive power of quantifying according to melanopic illuminance except in the rare case of exposure to narrowband very short wavelength light.

Consistent with the conclusions above, a recent evaluation of the other comprehensive action spectra for melatonin suppression from Brainard and colleagues (which used longer duration exposures—90 minutes)26,27 reported the responses were very well described by melanopic illuminance.19 Our own reanalysis of those data confirmed that conclusion (Figure 2A), with melanopic illuminance providing the best predictor of the magnitude of melatonin suppression across wavelengths (Figure 2B). Moreover, in this case, we found that simply expressing light intensity as melanopic illuminance allowed responses across wavelengths expected to maximally stimulate S-cones (440 nm), melanopsin (480 nm) and L/M-cones, respectively, (555 nm) to be described by a common function (Figure 2A; $F_{6,223} = 1.26$, $P = .28$). No other established photometric quantity had this ability (Figure 2B). This analysis therefore suggests that, for these 90 minutes exposures, melatonin suppression can be fully accounted for by melanopsin alone. Indeed, an unbiased assessment of the data reveals the best-fit opsin template has

![Figure 2](image-url)

**FIGURE 2** Melanopic illuminance dictates melatonin suppression during moderate to long nocturnal light exposures in subjects with dilated pupils. A: Data from26,27 showing mean ± SEM melatonin suppression evoked by 90 min monochromatic light pulses, quantified as melanopic EDI. B: $r^2$ for global 4-parameter sigmoid curve fits to data expressed as melanopic, photopic and other $\alpha$-opic illuminances. $F$ test comparisons indicated that responses to 440, 480 and 555 nm data could be accounted for by a single 4-parameter sigmoid when quantified as melanopic illuminance ($P = .28$) but not for any other metric. C: Goodness of fit for global best-fit 4-parameter sigmoid curves when data from26,27 were quantified according to $\alpha$-opic templates with arbitrary $\lambda_{max}$, revealing a best-fit at 482 nm. D: Data from29 showing melatonin suppression for individual subjects during the first or last quartile of a 6.5 h monochromatic light pulse, quantified as melanopic EDI. Solid curves show global best-fit 4-parameter sigmoid. E: $r^2$ for global 4-parameter sigmoid curve fits to data from29 expressed as melanopic, photopic or $s$-cone-opic illuminance across each quartile of the 6.5 h stimulus. Ability of a single curve to describe data for both wavelengths assessed by $F$ test as above. Note that goodness of fit in D and E is calculated based on individual subjects rather than group means hence $r^2$ values are expected to be lower than for (A and B) * and *** in (B and E) indicate $F$ test $P < .05$ and $P < .001$, respectively.
a λ_max of 482 nm (Figure 2C), essentially identical to that reported for melanopsin using other techniques.7,15,28,44 Also consistent with the conclusions above and previous retrospective analyses,19 data from a separate study,28 that compared melatonin suppression following 90 minutes exposures to fixed intensity narrowband lights of varying wavelength were similarly best explained when the stimuli were quantified as melanopic illuminance (Figure S2).

To further validate the conclusions above, and determine whether they extend also to longer duration light exposures, we also analysed data from another study that compared responses to short (460 nm) and long (555 nm) wavelength monochromatic light over 6.5 hours.29 That study reported changes in sensitivity to 555 nm, consistent with an influence of photopic illuminance that exponentially decayed over extended exposure. We found that, even for the first quartile of that 6.5 hours stimulus (a timeframe equivalent to that extended exposure. We found that, even for the first quartile of that 6.5 hours stimulus (a timeframe equivalent to that for the Brainard et al data26,27), melatonin suppression could be adequately described by melanopic illuminance alone (Figure 2D; F test: F_{3,42} = 1.5, P = .23). This was true also for rhodopic illuminance (which has most similar sensitivity to melanopsin) but not for photopic or any of the individual cone-opic illuminances (Figure 2E, Figure S3A,B). We did, however, note that the predictive power of melanopic illuminance increased for melatonin suppression across later components of the 6.5 hours stimulus and that this was accompanied by a decrease in the variance accounted for by photopic illuminance (Figure 2D,E, Figure S3A). On aggregate then, these data support the view that photopic illuminance may also influence melatonin secretion but that any such influence is restricted, with melanopic illuminance accounting for the vast majority of any response over all but very short durations.

3.2 Melatonin suppression in subjects with undilated pupils

While the most comprehensive evaluations of the spectral sensitivity of melatonin suppression have used subjects with dilated pupils, such measures are less directly relevant to the real-world situation where retinal illumination will be influenced by light-dependent pupil constriction. Moreover, given substantial data indicating that cone

**Figure 3** Melanopic illuminance dictates melatonin suppression during moderate to long nocturnal light exposures in subjects with undilated pupils. A. Data from30 showing mean ± SEM melatonin suppression evoked by polychromatic light exposure over each hour of a 3 h stimulus, quantified as melanopic EDI. B, r^2 for global 4-parameter sigmoid curve fits to data from30 expressed as melanopic, photopic and s-cone-opic illuminances. F test comparisons indicated that responses could be accounted for by a single 4-parameter sigmoid when quantified as melanopic or s-cone-opic but not photopic illuminance. C. Data from31 showing mean ± SEM melatonin suppression evoked by 90 min polychromatic light exposures. D, r^2 for global 4-parameter sigmoid curve fits to data from31 quantified as melanopic, photopic or other o-opic illuminances, with F test analysis as above. (E and F) Data from32-35 (≥4 h exposures) quantified as above. * and ** in (B and D) represent F test P < .05 and P < .01, respectively.
signals can influence pupil responses (including chromatic effects), it is theoretically possible that the data evaluated above do not fully recapitulate the spectral sensitivity of melatonin suppression under “natural” conditions. To assess this, we analysed data from a number of studies that assessed these properties in subjects with freely responsive pupils. As above, that any such effect of S-cones is both transient and only readily apparent for bright stimuli that are highly biased in favour of S-cones over melanopsin (such as narrowband 420 nm stimuli used in27).

3.3 Circadian phase resetting

Due to the technical challenges associated with directly assessing circadian responses, melatonin suppression has commonly been used as a proxy for the spectral sensitivity of the human circadian system. Given, however, mounting evidence that these aspects of biology are not always correlated, we next evaluated data that directly assessed the spectral sensitivity of circadian phase resetting. Although such data are scarce, Gooley et al29 report irradiance-response data for subjects with dilated pupils exposed to 6.5 hours of 460 or 555 nm monochromatic light. Consistent with the melatonin suppression data discussed above (Figure 2D,E), the measured phase shifts could be best predicted by melanopic illuminance (Figure 4A,B). In this case, however, even when quantified in that manner, we found a low probability that the data points for both wavelengths fell on a single curve (Figure 4A; F test: F2,428 = 4.6, P = .02). In particular, as noted by the authors, there was evidence for larger than expected responses to low intensity 555 nm light than one might predict based on melanopsin alone.
The data described above therefore imply either a positive contribution of photopic illuminance and/or a modulatory effect of blue-yellow colour (as identified in mice) to phase resetting under the conditions studied. Given the possibility of inter-individual differences in sensitivity (as reported for melatonin suppression), which could impact the apparent shape of the irradiance-response curves, any such conclusion should be treated with caution.

To further assess the sufficiency of melanopic illuminance as a predictor of circadian responses, we next identified studies that evaluated this in subjects with undilated pupils. Given the relative scarcity of such information, we combined data from studies using exposure durations from 2 to 6.5 hours. Importantly, the resulting data set was reliably accounted for by melanopic illuminance (Figure 4C), which once again provided the best descriptor of response sensitivity of any available metric (Figure 4D). When interpreting these data, it is important to note previous studies that demonstrate circadian phase resetting responses are sensitive to stimulus duration. The extent to which responses evoked by intensities in the range analysed here are sensitive to stimulus duration remains unknown, however. Accordingly, to provide independent validation of our analysis that aggregated data across studies employing different stimulus durations, we tested whether the derived relationship between melanopic illuminance and phase shift magnitude could predict the responses of another study which described irradiance-response data for 6.5 hours white light pulses. Importantly, there was a close correspondence between the phase shift predictions and the data reported by Zeitzer and colleagues (Figure 4E), with F test indicating that the two data sets could be adequately described by a common curve (F3,36 = 1.02, P = .40).

### Alerting responses

Another nonvisual response of significant practical interest is the ability of light to modulate alertness. While the sensory properties of alerting responses to light have not been studied in quite the same level of detail as for the other nonvisual responses discussed above, a few of the studies identified as part of this analysis also collected data on subjective...
sleepiness (assessed by KSS). Accordingly, we undertook a preliminary analysis to determine the extent to which these data were informative as to the spectral sensitivity of such responses. In total, we identified three studies\textsuperscript{32,33,37} that assessed subjective sleepiness under similar enough conditions that the data could be meaningfully aggregated for analysis of spectral sensitivity. These studies all tested the effects of various broadband lights during the late evening in subjects with undilated pupils. For analysis, we took KSS scores reported for 70-90 minutes prior to scheduled/habitual sleep time, at which point the subjects had been exposed to the test sources for at least 1 hour. In accordance with other data analysed above, we once again found that melanopic illuminance provided the nominally best predictor of the resulting data (Figure 5A) although, in this case, M-cone-opic and rhodopic illuminance provided near-identically good predictors of subjective sleepiness (Figure 5B, Figure S5A).

To evaluate the extent to which the relationship between melanopic illuminance and responses suggested by our analysis above was representative of alerting responses to evening/night-time light, we also compared this to a previously reported irradiance-response curve of alerting effects of white light\textsuperscript{40} (Figure 5C). The range of KSS scores reported in this latter study between bright and dim light exposure was substantially larger than in our aggregate analysis. Presumably, this at least partly reflects the quite different study conditions, with Cajochen and colleagues\textsuperscript{40} assessing subjective sleepiness during the late biological night (KSS scores collected 4-5.5 hours after the normal time of sleep onset). More significantly, however, re-scaling the data from our aggregate analysis to match\textsuperscript{40} also revealed a clear difference in sensitivity (Figure 5C; $F_{4,32} = 9.60$, $P < .0001$). In particular, while both data sets exhibited a very steep relationship between light intensity and subjective sleepiness, the half-saturating intensity was ~0.5 log units lower for the aggregate data set. This divergence cannot simply be explained by a difference in the photoreceptive systems contributing under the conditions studied, since sensitivity curves from the two data sets shift in a coordinated fashion for each of the illuminance quantities evaluated (eg Figure S5B). The difference in sensitivity might instead then reflect the greater “baseline” sleepiness under the conditions studied by Cajochen et al, such that more light was necessary to trigger an alerting effect. This conclusion remains highly tentative, however. More extensive evaluations of the sensitivity of alerting responses to light (and their relationship to time of day) will be required to address this issue in future.

### 3.5 Sensitivity range of melanopsin-driven nonvisual responses

Since melanopic illuminance provides the most reliable indicator of human nonvisual responses across a wide range

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**Figure 5** Spectral sensitivity of alerting responses to light. A, Data from\textsuperscript{32,33,37} showing subjective sleepiness (as measured by KSS scores obtained 70-90 min prior to scheduled sleep) across subjects exposed to various broadband sources for >1 h, quantified as melanopic, photopic and S-cone opic illuminance. Curves show best-fit 4-parameter sigmoid. B, $r^2$ for global 4-parameter sigmoid curve fits to data from\textsuperscript{32,33,37} expressed as melanopic, photopic or other $\alpha$-opic illuminance. C, White light irradiance-response relationship for subjective sleepiness from\textsuperscript{40} (mean KSS scores for individual subjects acquired 4-5.5 h after normal sleep onset) with best-fit 4-parameter sigmoid as above. Dashed line shows the melanopic illuminance-response curve from (A) re-scaled to match the relationship from\textsuperscript{40}.
FIGURE 6  Responses of the human circadian system are reliably predicted by melanopic illuminance and track levels of illumination corresponding to natural twilight transitions. A, Goodness of fit for single-opsin predictors of nonvisual response magnitude in subjects with dilated pupils (normalised to values obtained for melanopsin), analysed by one-sample t tests for difference from unity. Analysis based on data presented in Figures 1, 2, 4A-B and Figure S2 (where relevant data averaged across measurements in equivalent paradigms; ie Figures 1B,D, 2A,E and Figure S2). B, As above but for subjects with undilated pupils and over a less spectrally diverse range of light sources. Based on data in Figures 3, 4C-D and 5B. C, Dynamic range of best-fit curves to the relationship between melanopic EDI and nonvisual response magnitude for subjects with undilated pupils, with minimal responses (<25% of maximum) for stimuli < 1 lux and strong responses (>75% of max) for stimuli > 50 lux melanopic EDI. As expected, these values were shifted to higher levels for subjects with undilated pupils, with minimal responses (<25% of maximum) for stimuli < 4 lux and strong responses (>75% of max) for stimuli > 300 lux melanopic EDI. (Figure 6D). These values closely correspond to the typical melanopic illuminance of natural ambient illumination around dawn and dusk (Figure 6E; between sunrise/set and the boundary between civil and nautical twilight), consistent with the view this system is optimised to measure light intensities associated with day-night transitions. It is important to stress, however, that potentially significant nonvisual responses certainly can occur at light intensities lower than the thresholds specified above.

In data from subjects with freely responsive pupils (Figure 6D), we did not see any clear evidence for a systematic effect of exposure duration on the sensitivity of melatonin suppression. This analysis did not, however, include studies that used 30 minutes light exposures where previous analysis of responses under semi-naturalistic settings suggests ~10-fold lower sensitivity to melanopic illuminance (consistent with our analysis of data from subjects with dilated pupils; Figure 6C). At present, there are insufficient data available to determine whether a similar effect of stimulus duration applies to the sensitivity of circadian phase resetting or alerting responses to light (although the amplitude of circadian responses to bright light are certainly reduced for stimuli < 2 hours in duration\(^{31,52}\)).

When interpreting the data in Figure 6, it is also important to bear in mind recent data indicating significant inter-individual variation in sensitivity for melatonin suppression (~50-fold).\(^{38}\) All the analysis of group data reported here should, in principle, incorporate equivalent variability so that, for individual subjects, sensitivity may be compressed towards the upper or lower bounds of those indicated in Figure 6C.D.
variability impacts the other nonvisual responses assessed here awaits more definitive experimental data. It is interesting to note in this regard that the dynamic range for alerting responses is narrow compared with circadian phase resetting and melatonin suppression, potentially suggesting that the mechanisms which dictate sensitivity in this regard are different from the other nonvisual responses.

4 | CONCLUSIONS

Here, we provide, to our knowledge, the most comprehensive evaluation to date of the spectral sensitivity of human nonvisual responses to ocular light. Consistent with the results of previous studies, we find that melatonin suppression and circadian phase resetting can be accurately predicted and, in most cases fully accounted for, by melanopic illuminance. Moreover, we find good consistency in the range of melanopic illuminances over which these responses are evoked between different experimental paradigms. These data therefore provide strong support for the use of melanopic illuminance as the basis for guidelines that seek to regulate light exposure to benefit human health and to correspondingly inform the design and application of lighting and health-related devices/interventions (eg) for practical use and future scientific investigations.

It is important to note that the laboratory studies contributing to the present analysis all tested effects of light exposure during the evening/night. While it is reasonable to assume that the sensory properties described here apply also to circadian responses at other times (morning or daytime), this awaits definitive conformation. Further, the present analysis constitutes only a preliminary evaluation of the sensory properties of acute alerting responses to light. While understanding the sensory control of such responses is clearly of interest, there have not yet been any individual studies that have comprehensively addressed this (akin to the action spectra for melatonin suppression). Moreover, owing to the subjective measures most typically used to quantify alerting responses, there are significant challenges associated with meta-analysis of such data. Consistent with the results of the present analysis, there are certainly indications that melanopsin-selective modulations in light intensity can modulate alertness, both in the daytime and evening (but see). However, other recent retrospective analyses of the existing literature in this area suggest that alerting responses may be less reliably predicted by melanopic illuminance than for the other nonvisual responses studied here. Attaining a more detailed understanding of how light regulates alertness therefore remains a key question for the future.

We further stress here that, while the present analysis highlights melanopic illuminance as major contributor to nonvisual responses, these findings do not necessarily preclude a contribution from photoreceptors other than melanopsin. Indeed, we highlight some evidence above that cone signals can noticeably modulate nonvisual responses under certain conditions. To date, such effects have most clearly been demonstrated only under those circumstances that are of least relevance to nonvisual responses in the real-world (ie with dilated pupils, monochromatic light and/or short exposure durations). For example, in subjects with dilated pupils, there is evidence that 30 minutes exposures to narrowband light engage an S-cone contribution to melatonin suppression. However, such effects are not readily apparent in equivalent data obtained following longer exposures (eg) or in subjects with undilated pupils exposed to S-cone selective modulations in illuminance. In addition, while circadian phase resetting in subjects with dilated pupils is also not fully accounted for by melanopic illuminance, in this case, the data seem to reflect either a positive contribution of L/M cones or an inhibitory effect of S-cones at low light intensities.

Of note, those latter data are compatible with the possibility that the suppressive effect of “blue” colour on circadian responses recently identified in mice might also apply to humans. The extent to which colour signals modulate circadian responses in human subjects with undilated pupils is currently harder to ascertain, however. Certainly the analysis presented in the present study suggests melanopic illuminance is the dominant factor, as for melatonin suppression. Accordingly, a few studies have compared circadian responses to warm vs cool fluorescent light, none of which identified significant differences in phase resetting evoked by exposure to the two sources. Since the cool sources used in the latter studies had higher melanopic illuminance (~0.2 log units), those data are not incompatible with a modulatory effect of cones/colour but certainly suggest that any such effect is modest for the moderate to high melanopic illuminance conditions studied.

In summary, the possibility that more practically relevant contributions of cones to nonvisual responses might be identified in future should not be discounted. Nonetheless, while undoubtedly a simplification of the underlying biology, presently available data indicate that for most commonly encountered real-world situations (extended exposures to broadband light), melanopic illuminance provides a robust predictor of nonvisual responses with widespread utility.

ACKNOWLEDGEMENTS

The author thanks Prof. RJ Lucas for helpful comments on a draft of this manuscript.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

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.

ORCID
Timothy M. Brown https://orcid.org/0000-0002-5625-4750

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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**How to cite this article:** Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res*. 2020;69:e12655. https://doi.org/10.1111/jpi.12655