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A "Melanopic" Spectral Efficiency Function Predicts the Sensitivity of Melanopsin Photoreceptors to Polychromatic Lights

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Abstract Photoreception in the mammalian retina is not restricted to rods and cones but extends to a small number of intrinsically photosensitive retinal ganglion cells expressing the photopigment melanopsin. These mRGCs are especially important contributors to circadian entrainment, the pupil light reflex, and other so-called nonimage-forming (NIF) responses. The spectral sensitivity of melanopsin phototransduction has been addressed in several species by comparing responses to a range of monochromatic stimuli. The resultant action spectra match the predicted profile of an opsin:vitamin A–based photopigment (nomogram) with a peak sensitivity (λ_{max}) around 480 nm. It would be most useful to be able to use this spectral sensitivity function to predict melanopsin's sensitivity to broad-spectrum, including "white," lights. However, evidence that melanopsin is a bistable pigment with an intrinsic light-dependent bleach recovery mechanism raises the possibility of a more complex relationship between spectral quality and photoreceptor response. Here, we set out to empirically determine whether simply weighting optical power at each wavelength according to the 480-nm nomogram and integrating across the spectrum could predict melanopsin sensitivity to a variety of polychromatic stimuli. We show that pupillomotor and circadian responses of mice relying solely on melanopsin for their photosensitivity (*rd/rd cl*) can indeed be accurately predicted using this methodology. Our data therefore suggest that the 480-nm nomogram may be employed as the basis for a new photometric measure of light intensity (which we term "melanopic") relevant for melanopsin photoreception. They further show that measuring light in these terms predicts the melanopsin response to light of divergent spectral composition much more reliably than other methods for quantifying irradiance or illuminance currently in widespread use.

Key words melanopsin, photoentrainment, photometry, illuminance

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Biological photoreceptors are not equally sensitive to all wavelengths of light. As a result, light sources of equivalent power but different spectral composition can have vastly divergent biological effectiveness. The techniques of photometry provide methods of measuring light that take this fundamental problem into account. They are based around mathematical or optical filters that weight the energy at each wavelength according to the spectral sensitivity of the biological system under consideration. Integrating across all wavelengths then provides a 1-dimensional parameter that quantifies light in a biologically meaningful way. This conceptually simple methodology enables meaningful comparisons of light intensity provided by divergent light sources and under the wide variety of lighting situations encountered in the field. As such, it is an important enabling technology for scientific investigation and provides a basis for optimizing design and introducing standards in artificial and architectural lighting.

The most familiar, and widely used, photometric unit of measure for circadian researchers is "lux" or more correctly "photopic lux." It quantifies illuminance, the total power of light falling on a detector surface from any direction as perceived by a standard human observer according to the equation

$$P\phi = 683\int P(\lambda).V(\lambda)d\lambda, \quad (1)$$

where $P\phi$ is illuminance in lux, $P(\lambda)$ is spectral power (irradiance) in $W/m^2/nm$, and $V(\lambda)$ is the photopic spectral sensitivity function. $V(\lambda)$ describes the relationship between wavelength and perceived illuminance for a standard human observer under conditions favoring cone-based vision and is normalized to 1 at 555 nm, its sensitivity peak (λ_{max}).

As $V(\lambda)$ describes the spectral sensitivity of one aspect of human cone-based vision, photopic units have limited utility. In particular, they are not relevant for nonhuman species nor for other aspects of human vision. A specific example of the latter case is vision under low light levels when responses rely on rods. A different, scotopic, spectral sensitivity function ($V'(\lambda)$, with λ_{max} ~505 nm) has therefore been developed to replace $V(\lambda)$ in a version of equation 1, which allows illuminance to be measured in "scotopic lux" under these conditions.

The discovery of a third class of retinal photoreceptor in mammals provides a new challenge in light measurement. These melanopsin-expressing retinal ganglion cells (mRGCs) respond directly to light (Berson et al., 2002; Hattar et al., 2002). They are well known to drive a number of so-called nonimage-forming (NIF) light responses including circadian photoentrainment and pineal melatonin suppression, but extending also to the pupil light reflex, and modulations in diverse aspects of behavior and physiology (Bailes and Lucas, 2010). More recently, there has been growing evidence that they also contribute to conventional visual processes (Brown et al., 2010; Ecker et al., 2010). The spectral efficiency of mRGC photoreception in both rodents and humans has been estimated by comparing sensitivity to several near monochromatic stimuli over a range of wavelengths. The resultant action spectra match the predicted profile for an opsin:vitamin A–based photopigment with a peak sensitivity around 480 nm (Berson et al., 2002; Dacey et al., 2005; Gamlin et al., 2007; Lucas et al., 2001). The mRGC action spectra are significantly divergent from $V(\lambda)$, highlighting the inherent unsuitability of reporting light intensity in photopic lux for any response to which mRGCs may contribute.

It would be useful therefore to have a photometric unit of measure that was appropriate for melanopsin photoreception. The most straightforward approach to achieving this objective would be to use the mRGC action spectra as a melanopsin-relevant spectral sensitivity function (termed "melanopic" here), to be used in place of $V(\lambda)$ in a version of equation 1.

Evidence from a variety of sources, however, indicates that melanopsin may have an inherent, light-dependent, bleach recovery mechanism whose spectral sensitivity is long wavelength shifted with respect to the published mRGC action spectra (Rollag, 2008). In this case, wavelengths that on their own have limited ability to activate melanopsin could nonetheless influence mRGC responses to shorter wavelengths either by altering the rate at which bleached melanopsin is regenerated and/or reducing the lifetime of the photoactivated pigment. Here, we do not set out to resolve these theoretical possibilities but rather to empirically determine whether such effects preclude using a melanopic sensitivity function based upon the published action spectra to predict the melanopsin response to polychromatic light stimuli.

An important consideration in designing experiments to achieve this objective is that the mRGC light response itself is driven by the combined activity of extrinsic (originating in rod and cone photoreceptors) and intrinsic (melanopsin-derived) influences. As methods exist to measure light for rod and cone systems, our efforts here were focused on the melanopsin component of this response and thus required a method of studying melanopsin photoreception in vivo without

any influence of rods or cones. This has been achieved by assessing mRGC-evoked responses (pupillary constriction and circadian phase shifts) in transgenic mice (*rd/rd cl*) lacking rod and cone photoreception (Lucas et al., 1999).

MATERIALS AND METHODS

C3H/He *rd/rd cl* mice (Lucas et al., 1999) were bred at the University of Manchester, genotyped by PCR, and housed under a 12:12 light:dark cycle with food and water available ad libitum. All animals were >80 days of age at the time of recordings. Experiments conformed to the UK Animals (Scientific Procedures) Act of 1986.

Pupillometry was conducted as previously described (Lall et al., 2010). In brief, mice were dark adapted for at least 1 hour, and a topical mydriatic (0.1% atropine) was applied to their right eye. Consensual pupil responses were recorded by gently restraining the mouse so that the right eye sat at the aperture of an integrating sphere capable of providing full field illumination. The left eye was then illuminated with infrared (>900 nm) and monitored with a digital video camera connected to a personal computer. Pupil area was measured from captured video images using software developed in house. Light was provided by Xe-arc sources (Cairn Research Ltd., Kent, UK) fitted with a fast shutter (Uniblitz, Rochester, NY) and transmitted to the integrating sphere using a quartz fiber optic. Light intensity was adjusted using neutral density filters, and its spectral composition was altered with interference filters (Edmund Optics, York, UK). Monochromatic sources were 10-nm half-peak bandwidth.

For circadian experiments, mice were singly housed with free access to a running wheel, the rotations of which were monitored using the Chronobiology Kit (Stanford Software System, Santa Cruz, CA). Mice were maintained on a 12:12 LD cycle (white fluorescent light, color temperature of 3500 K; 804 $\mu W/cm^2$; 4337 m-lux) for at least 10 days to achieve stable entrainment before being released into constant darkness (DD). After at least 10 days in DD, light pulses were then administrated at CT16 (4 hours after activity onset), and the animals were returned to DD for another 10 days. Comparison of regression lines through the time of activity onset before and after the light pulses (excluding the first 3 days immediately after light pulse day) was used to calculate phase shifts. Light stimuli used in this experiment were applied in a specialized chamber with full internal reflectance connected using a quartz fiber optic to a Xe-arc light source (Cairn Research Ltd.). Lighting was controlled using an electric shutter (Uniblitz) and filtered using neutral density and interference filters (Edmund Optics).

Light Measurements and Manipulations

Spectral irradiance profiles for all light stimuli were measured between 300 and 800 nm in $W/m^2/nm$ using a spectroradiometer fitted with a cosine diffuser (Bentham Instruments, Reading, UK). These spectral irradiance profiles were used to calculate photopic illuminance (in photopic lux) according to equation 1, using the CIE (2007) 2° luminous efficiency function (http://www.cvrl.org/cvrlfunctions.htm) as $V(\lambda)$, and stimulus irradiance in photon flux (ϕ; photons/cm^2/sec) according to the equation

$$\phi = 5.03 \times 10^{15} \int P(\lambda).\lambda d\lambda, \quad (2)$$

where P is power in $W/cm^2/nm$, and λ = wavelength (nm).

Melanopic illuminance ($M\phi$) was calculated using a modified version of equation 1:

$$M\phi = 4557 \int P(\lambda).V^z(\lambda) d\lambda, \quad (3)$$

where $V^z(\lambda)$ is the proposed melanopic spectral efficiency function (note that the superscript z, denoting zeit [the German for time], is used because $V^m(\lambda)$ is already used in a different context). $V^z(\lambda)$ comprises the predicted spectral sensitivity profile of an opsin:vitamin A–based photopigment (Govardovskii et al., 2000), λ_{max} = 480 nm, expressed in relation to optical power rather than photon flux and corrected for prereceptoral screening by the lens/ocular media. For mice, this latter correction employed data for lens absorbance in pigmented (C57/bl6) animals shown to provide an adequate approximation of prereceptoral influences on cone spectral sensitivity (Jacobs and Williams, 2007). The corrected spectral sensitivity function used as $V^z(\lambda)$ is available online, at http://lucasgroup.lab.ls.manchester.ac.uk/research/measuringmelanopicilluminance/

The constant (4557) ensures that, for irradiance at 555 nm, 1 melanopic lux = 1 photopic lux for a standard human observer. This matches the approach used to relate scotopic and photopic lux. It also connects the proposed melanopic measure to the SI base unit of Cd (defined as the luminous intensity emitted by a source emitting monochromatic 540×10^{12} Hz radiation [555 nm in standard air] with a radiant intensity of 1/683 W/

steradian). Cd is further linked to photopic lux because the total luminous flux emitted by such a source over 1 steradian is 1 photopic lumen, and photopic lux = photopic lumen/m^2. In order to match melanopic and photopic lux at 555 nm, we therefore need to correct for the normalized sensitivity of the melanopic function at this wavelength. We estimated this as 0.14986 based upon a version of $V^z(\lambda)$ for a standard human observer (available at http://lucasgroup.lab.ls.manchester.ac.uk/research/measuringmelanopicilluminance/; see also the supplementary online material for this article) produced by correcting the 480-nm nomogram for human prereceptoral filtering (Stockman and Sharpe, 2000). As there are 683 photopic/scotopic lumens per watt at 555 nm, the constant in equation 3 must be 683/0.14986 or 4557 in order for this to be true also for melanopic lumens.

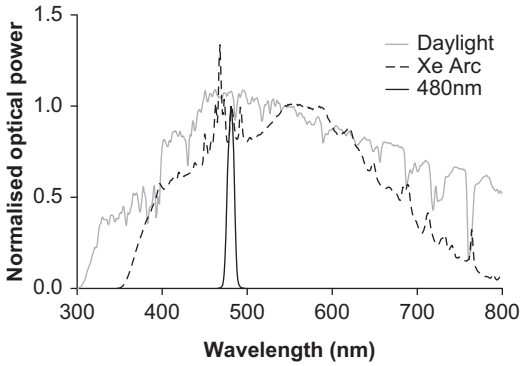

Figure 1. Normalized spectral irradiance profiles for the unfiltered Xe-arc and 480-nm monochromatic stimuli used in these experiments. For comparison, the spectral irradiance of the sky under thick, low clouds after recent light rain at midday in Manchester, UK (53° 21′ N 2° 16′ W; elevation of 78 m), 3 weeks after autumnal equinox is shown. Spectra normalized for optical power = 1 at peak wavelength for 480 nm and arbitrarily at 546 nm for Xe-arc and daylight spectra.

Data Handling and Statistical Analysis

Measurements of circadian and pupillomotor responses elicited by spectrally distinct light sources were undertaken in a number of discrete studies. Within each of these studies, exposure to 2 or more different light conditions was alternated in order to avoid any order effects. For this reason, statistical comparisons of response amplitude/sensitivity between light sources were restricted to within each of these studies.

Where appropriate, pupillometry data were fitted with a sigmoidal function constrained for upper asymptote <1 and lower asymptote >0. An *F* test statistic was used to determine whether pupil responses from diverse light sources could be fit with a common curve.

Some simple modeling was undertaken on the pupil responses to 480-nm, unfiltered Xe-arc, <500-nm, and >500-nm stimuli in order to provide an unbiased estimate of the spectral sensitivity of the photoreceptor mediating pupil constriction in *rd/rd cl* mice. To this end, we calculated the effective photon flux in each light stimulus for a series of putative opsin proteins, whose spectral sensitivity conformed to the nomogram (Govardovskii et al., 2000) with peak sensitivities between 420 and 540 nm and corrected for effects of lens absorption (Jacobs and Williams, 2007). Because our data comparing the narrow-band 480-nm light with a full-spectrum xenon arc source (study 1) and with short/long pass–filtered versions of this stimulus (study 2) were conducted in separate, carefully balanced experiments, we initially analyzed these data separately. For each study, we determined the EC_{50} of the sigmoid curve that best fit all the data points (GraphPad Prism, La Jolla, CA), corrected according to the sensitivity of the hypothetical opsin and irrespective of the stimuli that generated them. Subsequently, we adjusted the effective intensities of the 480-nm and xenon arc data from study 1 so that the estimated EC_{50} matched those calculated for the stimuli in study 2 and the same arbitrary opsin. This adjustment accounted for interindividual variability in intensity response relationships. Finally, we determined the sigmoid relationships that best fit all the intensity-corrected data points from both studies across the series of hypothetical opsin proteins. The *F* test probabilities that these fits better explained the intensity response relationships than separate sigmoidal functions for each visual stimulus were then used to describe the likelihood that opsin proteins with a given peak sensitivity accounted for the data.

RESULTS

Broad-Spectrum "White" Light

We first determined the suitability of the melanopic function for predicting melanopsin-driven responses to a broad-spectrum "white" light. We chose to use a Xe-arc lamp as a "white" source because its spectral composition provides the closest approximation of natural daylight that can be readily achieved in the laboratory (Fig. 1). We set out then to compare intensity response curves for pupil responses driven by this broad-spectrum source or a near monochromatic

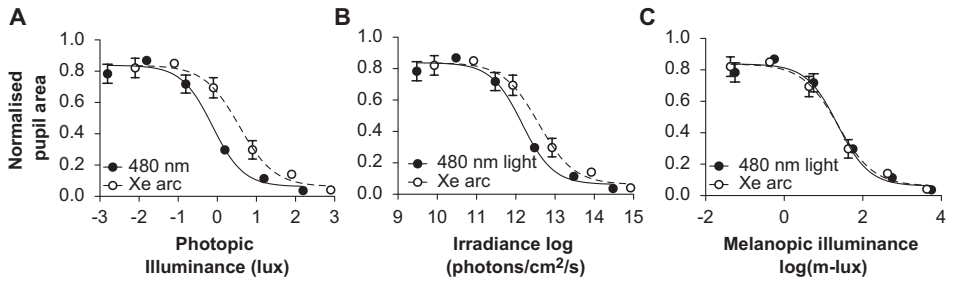

Figure 2. Stimulus response functions for the consensual pupil light reflex elicited by the unfiltered Xe-arc source and a monochromatic 480-nm stimulus. Data show mean ± SEM pupil area ($n = 5$) normalized to the dark-adapted size for each animal, plotted against light intensity quantified as photopic illuminance (A), irradiance (B), or melanopic illuminance (C).

(10-nm half-peak bandwidth) 480-nm light (Fig. 1) in mice lacking rod and cone photoreceptors (*rd/rd cl*) (Lucas et al., 1999).

We started by determining whether the unit of light intensity most widely used in chronobiology (photopic lux) accurately predicted the mouse's response to these 2 light stimuli. We found that it did not. Thus, stimulus response curves for pupil constriction were highly divergent when plotted as a function of photopic illuminance (*F* test of the probability that data could be fit by a single curve, $p < 0.01$) (Fig. 2A). Another widely used method for quantifying light in chronobiology is to use radiometric units. These have the benefit of making no assumption about the spectral sensitivity of the biological detector driving the response. We found that this approach was also inappropriate for predicting pupil responses to these 2 light sources. Thus, expressing stimulus intensity in photon flux (photons/cm^2/sec) (Fig. 2B), or indeed optical power (μW/cm^2) (not shown), did not allow the 2 datasets to be fit by a single curve (*F* test, $p < 0.05$). By contrast, the 2 stimulus response relationships converged when light intensity was expressed in the proposed new measure of melanopic illuminance (*F* test, $p > 0.05$) (Fig. 2C).

Melanopic illuminance proved equally suitable for predicting the ability of these 2 light sources to elicit circadian phase shifts. Thus, we found that stimulus response curves for the phase delay elicited by 15-minute pulses of either 480-nm or unfiltered "white" light (Fig. 1) were superimposed when light intensity was quantified in melanopic lux, denoted as m-lux (Fig. 3).

"Blue" and "Orange" Lights

To further validate the melanopic function, we tested whether it could also be used to predict responses to polychromatic lights that were heavily enriched for either the short or long wavelength portions of the visible spectrum. To this end, we compared responses to the Xe-arc source when fitted with monochromatic 480-nm band-pass, 500-nm short-pass, or 500-nm long-pass filters (Fig. 4). Stimulus response curves for the pupil constriction elicited by these 3 lights were highly divergent when light intensity was expressed in terms of either photopic illuminance (*F* test, $p < 0.001$) (Fig. 5A) or photon flux ($p < 0.05$) (Fig. 5B). Once again, however, the curves became superimposed when the melanopic illuminance measure was employed (*F* test, $p > 0.05$) (Fig. 5C).

Rather than construct full-irradiance response curves for circadian entrainment for each of these 3 lights, we picked a single intensity for the 480-nm stimulus predicted to drive a subsaturating response. We then set the irradiance of the 2 polychromatic lights to give an equivalent melanopic illuminance (2.5 m-lux). We found that these stimuli indeed drove phase delays of equivalent magnitude (58 ± 8, 56 ± 19, and 53 ± 8 [mean ± SEM] minutes for 480 nm, <500 nm, and >500 nm, respectively; 1-way ANOVA, $p > 0.05$; $n = 5$), even though they were substantially divergent in terms of photon flux (10.9, 11.3, and 12.3 log photons/cm^2/sec) or photopic illuminance (0.07, 0.07, and 2.5 photopic lux for 480 nm, <500 nm, and >500 nm, respectively).

Modeling the Combined Data

Having confirmed that the proposed melanopic function provided a satisfactory method for predicting melanopsin sensitivity to spectrally diverse stimuli, we turned to a more unbiased assessment of the most appropriate method for quantifying these lights. To this end, we calculated normalized spectral irradiance profiles for all light sources according to the spectral sensitivity functions for opsin:vitamin A–based photopigments with λ_{max} in the 420- to 540-nm range. These were then integrated across wavelengths to calculate the "weighted" irradiance of each light condition as experienced by each putative pigment. We then used an *F* test statistic to determine the probability (p) that calculating weighted irradiance in this way allowed the irradiance response relationships presented in Figures 2 and 5 to be fit with a single curve. We found

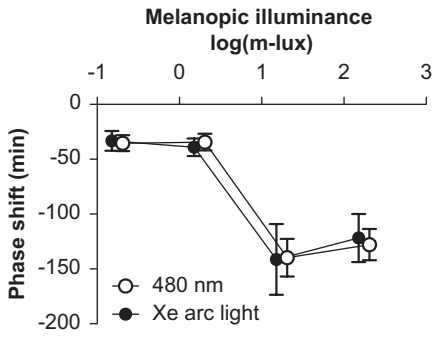

Figure 3. Stimulus response functions for circadian phase delays elicited by 15-minute pulses of the unfiltered Xe-arc source and a monochromatic 480-nm stimulus. Data show mean ± SEM phase shift (n = 5 for the highest 2 illuminances, 9 for the third, and 3 for the lowest).

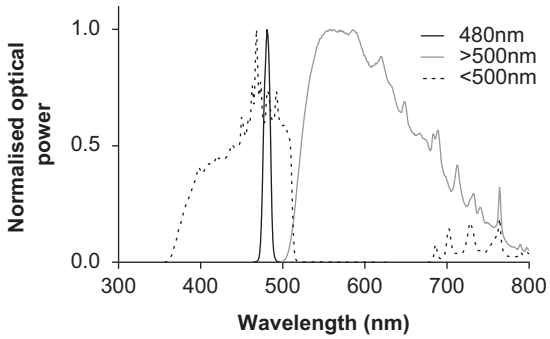

Figure 4. Normalized spectral irradiance profiles for <500-nm, >500-nm, and 480-nm monochromatic stimuli. Spectra normalized for optical power = 1 at peak wavelength.

that the relationship between F test–derived probability (p) and putative photopigment λ_{max} could be fit with a Gaussian function (Fig. 6), peaking close to 480 nm (484 nm). This unbiased assessment then confirms that the λ_{max} 480-nm opsin nomogram represents the most appropriate, currently available basis for quantifying these spectrally diverse lights with respect to their ability to activate melanopsin.

DISCUSSION

We show that a derivative of the λ_{max} = 480-nm opsin nomogram can be used as a "melanopic" spectral efficiency function to accurately predict the response of mouse melanopsin to diverse polychromatic stimuli. Our data further confirm that this goal cannot be achieved by measuring light intensity in either the radiometric (uW/cm^2; photons/cm^2/sec) or photometric (photopic lux) units currently in common usage for circadian studies. We therefore propose that the method presented here for quantifying melanopic illuminance be considered wherever the activity of mRGCs may contribute to experimental outcome.

Our proposed melanopic function has been shown to predict the sensitivity of *rd/rd cl* mice to a range of broad-spectrum "white" and "colored" lights. This provides confidence of its suitability for most commonly encountered light conditions. Nevertheless, by necessity, we have employed a limited array of wavelength conditions. It remains formally possible that the melanopic function would fail to predict melanopsin responses to lights whose spectral composition falls outside those presented here. In time, modifications to the melanopic measurement system may prove necessary to account for these limitations. Similarly, while at present our data do not support the hypothesis that mouse melanopsin is a bistable pigment in vivo, nor do they exclude this possibility.

An important practical question is whether the melanopic function would work as effectively for melanopsin in humans and other species as it does for mice. The first consideration in addressing this question is whether there is much interspecies variation in melanopsin spectral sensitivity. The currently available data suggest that there is not. Thus, melanopsin spectral sensitivity peaks near 480 nm not only in mice but also in nonhuman primates (Dacey et al., 2005), rats (Berson et al., 2002), and even chickens (Torii et al., 2007). Early action spectra for melatonin suppression in humans indicated a somewhat shorter λ_{max} (Brainard et al., 2001; Thapan et al., 2001), but more recent analyses of those data (Foster and Hankins, 2002) and others (Gamlin et al., 2007; Hankins and Lucas, 2002) support the more parsimonious hypothesis that human melanopsin too peaks around 480 nm.

A more substantive consideration in adopting the melanopic function ($V^z(\lambda)$) to predict responses in other species is that of prereceptoral filtering. The location of mRGCs on the inner layers of the retina means that melanopsin will be subject to the same wavelength-dependent filtering by the ocular media as the conventional rod and cone photoreceptors. The nature and extent of this filtering varies across species. In the case of mice, transmission is fairly neutral through the human visible spectrum and down into the UV (Jacobs and Williams, 2007), making our corrected melanopic function little different from the 480-nm nomogram. Rat and Syrian hamster lenses have similar transmission characteristics (Lei and Yao, 2006; Williams and Jacobs, 2008), implying that there would be little danger in using the mouse $V^z(\lambda)$ also for these common laboratory rodents.

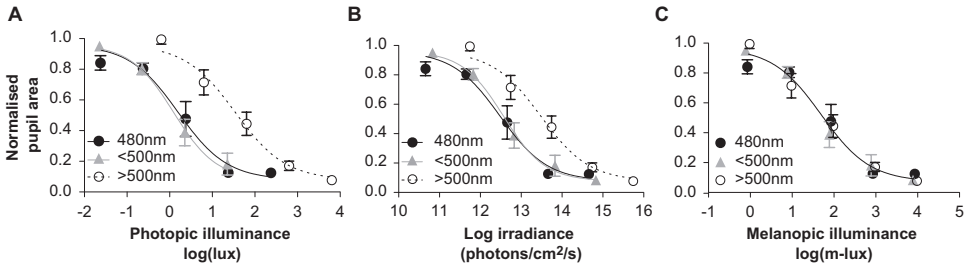

Figure 5. Pupillomotor responses to 480-nm, "blue" (<500 nm), and "orange" (>500 nm) stimuli. Data show mean ± SEM pupil area ($n = 5$) normalized to the dark-adapted size for each animal, plotted against light intensity quantified as photopic illuminance (A), irradiance (B), or melanopic illuminance (C).

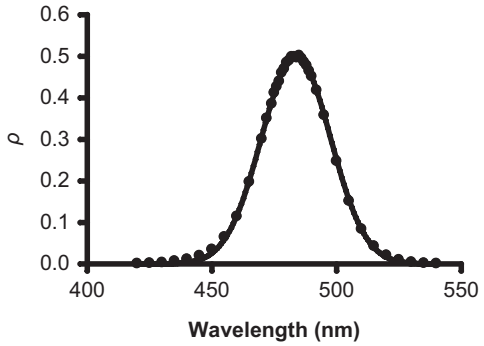

Figure 6. The spectral sensitivity template of a putative opsin pigments with λ_{max} around 480 nm provides the best basis for predicting the melanopsin response to spectrally diverse light sources. The *F* test probability (*p*) that stimulus response relationships for pupillary responses in *rd/rd cl* mice to all monochromatic and polychromatic stimuli used here can be fit with a single curve when spectral irradiance profiles are weighted according to the spectral sensitivity of a family of putative opsin photopigments with λ_{max} in the range of 400 to 550 nm. The data are fit with a Gaussian distribution peaking at 484 nm.

By contrast, anterior components of the human eye (especially the lens and luteal pigment) selectively absorb UV and shorter wavelength visible light, an effect that is positively correlated with age (Van de Kraats and van Norren, 2007). As mRGCs lie outside of the macular, luteal pigment is not a significant concern, but lens absorption should be taken into account. In developing equation 3, a putative human version of $V^z(\lambda)$ was generated (available online, at http://lucasgroup.lab.ls.manchester.ac.uk/research/measuringmelanopicilluminance/). This was based upon published data for prereceptoral filtering in young adults (Stockman and Sharpe, 2000) and, in this regard, has a similar validity as the photopic $V(\lambda)$, which also reflects the experience of a standard observer. Modulations may be considered when working with very old or young subjects (Van de Kraats and van Norren, 2007).

In employing melanopic quantities, it is important to recognize that they provide a method for predicting melanopsin photoactivation rather than the magnitude of any particular behavioral or physiological response downstream of mRGCs. In the rod- and cone-less mice used for these experiments, this distinction is unimportant because all evoked responses originate with melanopsin phototransduction. However, in animals with an intact visual system, downstream responses may additionally be influenced by rods and cones. This places limits on the suitability of melanopic measures.

The low sensitivity of melanopsin phototransduction (Berson et al., 2002; Dacey et al., 2005; Do et al., 2009; Schmidt et al., 2008) implies that measuring light in melanopic units is inappropriate under low light conditions. The data presented here, and published reports (Hattar et al., 2003; Lucas et al., 2003), indicate that rod-less + cone-less (*rd/rd cl*) mice do not show reliable pupillomotor or circadian responses to corneal irradiances $<10^{11}$ photons/cm^2/sec at approximately 480 nm (1.8 m-lux). This figure is broadly consistent with the lowest estimates for the melanopsin response threshold in vitro (Berson et al., 2002; Dacey et al., 2005; Do et al., 2009; Schmidt et al., 2008), allowing for the distinction between retinal and corneal irradiance (Lyubarsky et al., 2004). The threshold corneal irradiance for melanopsin activation may, however, be somewhat higher in wild-type mice because these animals have dim-light pupil constriction that would reduce the light reaching mRGCs (Lucas et al., 2001). Assuming that human melanopsin has similar sensitivity, the threshold corneal irradiance in our own species would be at least 10 times greater to allow for the larger ratio of retinal to pupil area. A reasonable assumption is therefore that melanopic units should not be used to describe corneal illuminance less than approximately 1.8 m-lux for mice and approximately 18 m-lux in humans. As rods probably dominate NIF responses under those conditions (Altimus et al., 2010; Lall et al., 2010; McDougal and Gamlin, 2010), scotopic (or mesopic) spectral efficiency functions should provide a more reliable predictor of NIF responses evoked by such relatively dim lights.

The situation at higher irradiances, within the melanopsin sensitivity range, is more complex. Cones remain sensitive and support form vision under even the brightest illumination. In species in which cone vision is spectrally quite distinct from that of melanopsin (e.g., humans), cones could therefore strongly influence the spectral sensitivity of mRGC-driven responses. In these cases, the degree to which the melanopic spectral efficiency function matches that of evoked responses will thus depend on the extent to which cones contribute to NIF vision. Experiments from a number of species report excitatory (and perhaps inhibitory) cone input to the mRGC pathway (Dacey et al., 2005; Dkhissi-Benyahya et al., 2007; Gooley et al., 2010; Lall et al., 2010; Schmidt and Kofuji, 2010). Indeed, such effects provide a plausible explanation for failures to predict the efficiency of various polychromatic lights for melatonin suppression and other biological responses in humans using the 480-nm nomogram (Figueiro et al., 2005; Revell et al., 2010; Revell and Skene, 2007).

Rea et al. (2005) proposed a method to address this problem by combining spectral efficiency profiles for melanopsin, rods, and cones to provide a composite function that can explain melatonin suppression data from humans exposed to a variety of monochromatic and polychromatic lights. Such an approach has obvious attractions. However, it seems increasingly unlikely that such a single method could adequately describe the spectral sensitivity of all mRGC-driven responses under all conditions. Thus, it seems that the relative contribution of cone and melanopsin photoreception to evoked responses is fundamentally plastic. We know that it is dependent upon the nature of light exposure (Dkhissi-Benyahya et al., 2007; Gooley et al., 2010; Lall et al., 2010). Moreover, there is emerging evidence for different mRGC classes with distinct intraretinal connections (Schmidt and Kofuji, 2010). Insofar as these classes are likely to combine rod, cone, and melanopsin inputs differently, the impact of cones on the spectral sensitivity of responses driven by them could also be distinct. Finally, the discovery that some neurons within the brain respond robustly to melanopsin but not cone activation (Brown et al., 2011) confirms that central processing could also reduce cone influence for some responses.

Until a consensus forms on the factors determining cone contributions to NIF vision, it will not be possible to provide a "one-size-fits-all" correction to the melanopic function to take account of cone influence. In the meantime, the utility of melanopic quantities resides in their status as the best currently available method of predicting melanopsin photoactivation. In this regard, there seems a clear benefit to including a melanopic quantity in the array of methods available to quantify light for human subjects. This would facilitate comparisons of data across laboratories and relate them to field conditions. It could also contribute to updating standards for architectural and artificial lighting. Today's lighting standards are based on visual aspects of light only. However, first attempts are being made to define quantities that concern the biological NIF potency of different kinds of light (DIN V 5031-100, 2009). A melanopic quantity could be very valuable in this approach.

Recording spectral irradiance and calculating melanopic illuminance need not be onerous. A range of low-cost spectrometers is available that can be used to generate spectral irradiance profiles. Methods for using these to calculate melanopic illuminance, along with the proposed melanopic functions (see supplementary online material), have been posted at http://lucasgroup.lab.ls.manchester.ac.uk/research/measuringmelanopicilluminance/. We will endeavor to update this resource in light of any future refinement in our understanding of melanopsin photobiology.

In summary, we validate a method for measuring broad-spectrum (including white) light in a manner that accurately predicts its effects on melanopsin photoreception. The melanopic spectral efficiency function underpinning this methodology ($V^z(\lambda)$) may require modification in due course to take account of future developments. Nevertheless, our data provide confidence that the current iteration is suitable for most commonly encountered light conditions. Moreover, as a method of predicting melanopsin activation, it represents a clear advance over currently accepted approaches to quantifying light in chronobiology and/or the lighting industry. If widely adopted, this methodology would therefore facilitate biologically meaningful comparisons of light intensity across laboratories and between laboratory and the field (supplementary online material).

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CONFLICT OF INTEREST STATEMENT

The authors have declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Philips Lighting employs Luc Schlangen and has provided research support to Robert Lucas and Victoria Revell.

NOTE

Supplementary material for this article is available on the *Journal of Biological Rhythms* website at http://jbr.sagepub.com/supplemental.

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