PREDICTING HUMAN NOCTURNAL NON-VISUAL RESPONSES TO MONOCHROMATIC AND POLYCHROMATIC LIGHT WITH A MELANOPSIN PHOTOSENSITIVITY FUNCTION

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ABSTRACT

The short-wavelength (blue) light sensitivity of human non-visual responses is recognised as being melanopsin-based. However, whether melanopsin is the sole factor in determining the efficacy of a polychromatic light source in driving non-visual responses remains to be established. Monochromatic (λ_{max} 437, 479 and 532 nm) and polychromatic (colour temperature: 4000 K and 17000 K) light stimuli were photon matched for their predicted ability to stimulate melanopsin, and their capacity to affect nocturnal melatonin levels, auditory reaction time and subjective alertness and mood was assessed.

Young, healthy male participants aged 18 – 35 years (23.6 ± 3.6 yrs; mean ± SD; n = 12) participated in 12 overnight sessions that included an individually timed 30 min light stimulus on the rising limb of the melatonin profile. At regular intervals before, during and after the light stimulus subjective mood and alertness were verbally assessed, blood samples were taken for analysis of plasma melatonin levels and an auditory reaction time task (psychomotor vigilance task: PVT) was performed. Proc GLM repeated measures ANOVA analysis revealed that significantly lower melatonin suppression was observed with the polychromatic light conditions (4000 K and 17000 K) compared to the 'melanopsin photon-matched' monochromatic light conditions ($p < 0.05$). In contrast, whilst subjective alertness significantly increased across the course of the light exposure it was significantly lower under the 479 nm monochromatic light condition compared to the 437 and 532 nm monochromatic and both polychromatic light conditions.

The demonstration that the melatonin suppression response to polychromatic light was significantly lower than predicted by the melanopsin photosensitivity function suggests that this function is not the sole consideration when trying to predict the efficacy of broadband lighting. The different spectral sensitivity of the subjective alertness and melatonin suppression responses may imply a differential involvement of the cone photopigments. An analysis of the photon densities in specific wavelength bands for the polychromatic lights used in this and our previous study suggests that the spectral composition of a polychromatic light source, and particularly the very short wavelength content, may be critical in determining response magnitude for the non-visual effects of nocturnal light.

INTRODUCTION

Non-visual responses to light in humans are short-wavelength (blue light) sensitive (Brainard et al., 2001; Thapan et al., 2001; Lockley et al., 2003; Cajochen et al., 2005; Revell et al., 2005; Lockley et al., 2006; Revell et al., 2006) and this is attributed to the melanopsin photopigment that is expressed in the photosensitive retinal ganglion cells (pRGCs) (Provencio et al., 2000; Gooley et al., 2001; Berson et al., 2002). Work in transgenic mice has demonstrated that whilst melanopsin-pRGCS are sufficient to sustain non-visual responses to light (Lucas et al., 2001; Hattar et al., 2003) it appears that both the rod and cone visual photopigments also contribute (Ruby et al., 2002; Lucas et al., 2003) primarily via the pRGCs (Guler et al., 2008). The relative contribution of rods, cones and melanopsin to non-visual responses appears to be context dependent and varies with the nature of the light stimulus (Lall et al., 2010).

Lighting in industrial, clinical and home environments needs to optimally stimulate both the visual and non-visual systems and hence, broadband (400 – 700 nm) polychromatic lighting is appropriate. However, to date, lighting has not been optimised for the non-visual system and this will likely be a highly complex procedure. Firstly, it has been demonstrated, via physiological and behavioural studies in transgenic mice lacking specific photopigments, that the three photopigment classes contribute differentially to different non-visual responses (Lucas et al., 2003; Altimus et al., 2008; Lupi et al., 2008; Thompson et al., 2008; Tsai et al., 2009; Lall et al., 2010). Thus, optimal light for one situation/environment/population may not be effective or appropriate for another. Secondly, there may be a time of day dependence to photopigment contribution (Tsai et al., 2009) which could reflect diurnal rhythms in retinal processing and/or gating of photopigment signals to the relevant brain areas. Finally, melanopsin appears to be a bistable photopigment and can form stable associations with both 11-*cis* (R, rhodopsin) and all-*trans* (M, metarhodopsin) isoforms of retinaldehyde and utilise light to transition between states. Whilst the photosensitivity function ($R \rightarrow M$) of melanopsin is most sensitive to short wavelength blue light the spectral sensitivity of the photoisomerase function ($M \rightarrow R$) is currently unknown although both *in vitro* and *in vivo* studies implicate longer wavelengths (540 – 620 nm) (Melyan et al., 2005; Mure et al., 2007; Mure et al., 2009). Such bistable properties of melanopsin may also influence non-visual responses to broadband lighting.

Previously we have demonstrated that the melatonin suppression response to an ultra high-pressure mercury polychromatic light was significantly greater than would be predicted by a melanopsin photosensitivity function with λ_{max} 480 nm (Revell and Skene, 2007). We postulated that this unexpected effect could be due to a stimulatory effect of the wide range of wavelengths present in the polychromatic light source either via activation of the visual photopigments or the melanopsin photoisomerase function.

The aim of the current study was to assess whether the melanopsin photosensitivity function (λ_{max} 480 nm) could predict acute non-visual responses to monochromatic and polychromatic light with different spectral compositions. It was hypothesised that if melanopsin was the sole contributor to nocturnal non-visual responses then light stimuli matched for melanopsin efficacy would produce the same magnitude responses. Two polychromatic light sources (colour temperature: 4000 K and 17000 K) and three monochromatic lights, administered singly and in combination with 479 nm, were matched for predicted melanopsin efficacy and tested. The monochromatic light conditions were chosen to be close to the wavelengths of maximal sensitivity of melanopsin (λ_{max} 479 nm) and also the S-cones (λ_{max} 437 nm) and the M-cones (λ_{max} 532 nm) to try to discern any influence of these visual photopigments.

METHODS

Subjects

Healthy male participants (n = 12) aged 18 – 35 years (23.6 ± 3.6 yrs; mean ± SD) completed 12 overnight laboratory sessions in a within-subject, randomised, crossover design. Participants were non-smokers, free from drugs of abuse and prescription medication, and reported no medical disorders. All participants underwent a full ophthalmic examination by a registered optician; participants were not colour blind and were free of ocular disorders. They had regular sleep schedules and reported no sleep disorders (Pittsburgh Sleep Quality Index ≤ 5). All participants were neither morning nor evening types according to the Horne-Östberg morningness-eveningness questionnaire (HÖ MEQ score: 50.9 ± 3.6). The study was approved by the University of Surrey Ethics Committee and all participants gave written consent before the study began. All procedures were conducted in accordance with the Declaration of Helsinki and conformed to international ethical standards (Portaluppi et al., 2008).

Pre-study measurements

From 10 days prior to the first in-laboratory session and for the duration of the study participants were required to maintain a strict sleep/wake schedule and regulate their alcohol, caffeine and non-steroidal-anti-inflammatory drug intake, similar to that previously described (Revell and Skene, 2007). In addition, participants were also required to complete a 48 h urine collection (4 h collection periods, 8 h overnight) for measurement of the major urinary metabolite of melatonin, 6-sulphatoxymelatonin (aMT6s). Cosinor analysis of the aMT6s rhythm was performed to determine the peak time of aMT6s production. The light exposure was then individually timed to occur on the rising phase of the melatonin rhythm.

In-laboratory protocol

The protocol used was similar to that previously described (Thapan et al., 2001; Herljevic et al., 2005; Revell and Skene, 2007). All participants completed 12 overnight in-laboratory sessions (19:00 – 07:00 h; October 2007 – April 2008) with a 2 h experimental period between 23:00 and 02:30 h. Within this period there was a 30 min light exposure for 10 sessions; the remaining two sessions were baseline, no-light conditions. Data from two of the light conditions tested during this study protocol were not applicable to the hypotheses tested here and are thus not presented. The sessions were run in blocks of three consecutive nights with blocks separated by at least four days. Nights one and six were always no-light conditions and the ten light conditions were randomised amongst the remaining nights.

Posture and environmental lighting were strictly controlled throughout each study session as previously described (Revell and Skene, 2007). Blood samples were taken at 15 min intervals from 30 min before (-30) until 90 min after (90) the start of the light exposure for measurement of plasma melatonin levels by radioimmunoassay (Fraser et al., 1983). Participants were asked to verbally rate their mood and alertness levels at 15 min intervals from 15 min before (-15) until 45 min after (45) the start of the light exposure. Subjective mood and alertness were assessed using the Karolinska Sleepiness Scale (KSS) (Akerstedt and Gillberg, 1990) and four 9 point mood and alertness scales (Revell et al., 2006; Lockley et al., 2008; Sletten et al., 2009). A 10 min auditory test on a psychomotor vigilance task (PVT) monitor was utilised to assess reaction time before, during and after the light exposure. Auditory tests were scheduled to start 10 min before (-10), 20 min after (20) and 50 min after (50) the start of the light exposure. The same protocol was conducted on all study sessions.

Light exposure

Participants were administered pupil dilator (Minims Tropicamide 1.0%, Chauvin Pharmaceuticals, Romford, UK) in each eye 90 min before the start of the 30 min light exposure. The light was administered using a sphere (45 cm diameter, Apollo Lighting, Leeds, UK) coated with a white reflectance paint (WRC-680 Labsphere, Pro-Lite Technology, Bedfordshire, UK). For the monochromatic light conditions the spheres were illuminated with a fibre optic cable connected to an ultra high-pressure mercury lamp (Focus 100LS3, 100 W, Philips Lighting, Eindhoven, The Netherlands). For the two polychromatic light conditions the spheres were illuminated with specially housed fluorescent tubes with colour temperature of 17000 K or 4000 K (Philips Lighting).

Light irradiances were measured with a calibrated radiometer (R203, Macam Photometrics Ltd., Livingston, Scotland) and the spectral distribution of the lights was measured with a calibrated spectrometer (Ocean Optics BV, Dunedin, Florida, USA). The monochromatic light conditions were produced using narrow bandwidth interference filters (Coherent Ealing Europe Ltd., Watford, UK) with λ_{max} 440, 480 and 535 nm (half maximal bandwidth $\lambda_{0.5}$ 10 nm). *In situ* λ_{max} of the filters were shifted to 437, 479 and 532 nm, respectively. The spectral power densities of the light conditions are shown in Figures 1a and 1b.

Light conditions and calculations

The parameters for each light condition are given in Table 1. The predicted efficiency of the light stimulus to stimulate melanopsin was estimated as previously described (Revell and Skene, 2007). Briefly, this was calculated as the number of effective photons integrated for wavelength and melanopsin spectral sensitivity, based on a Dartnall nomogram (Dartnall, 1953) with λ_{max} 480 nm. The light conditions were matched so that they delivered the same number of 'melanopsin-stimulating' photons at the cornea although the total photon flux differed between light conditions. The monochromatic and polychromatic light conditions were matched at 2.5×10^{13} 'melanopsin-stimulating' photons/cm^2/sec whilst the combined monochromatic conditions were matched at 5.0×10^{13} 'melanopsin-stimulating' photons/cm^2/sec. In addition, the photon flux in 50 nm wavelength blocks was calculated for the light conditions used in the current study and for the polychromatic light used in our previous study (Revell and Skene, 2007) to provide assistance in interpretation of the results (Table 2).

Table 1. Irradiance and photon flux parameters for each of the light stimuli.

Light condition	**'Melanopsin-stimulating' photon density (photons/cm^2/sec)**	**Total photon density (photons/cm^2/sec)**	**Irradiance (μW/cm^2)**
437 nm	2.5×10^{13}	4.2×10^{13}	19.1
479 nm	2.5×10^{13}	2.5×10^{13}	10.4
532 nm	2.5×10^{13}	6.4×10^{13}	23.8
4000 K	2.5×10^{13}	1.0×10^{14}	36
17000 K	2.5×10^{13}	5.2×10^{13}	20.3
437 + 479 nm	$2.5 \times 10^{13} + 2.5 \times 10^{13}$	6.7×10^{13}	29.5
479 + 479 nm	$2.5 \times 10^{13} + 2.5 \times 10^{13}$	5.0×10^{13}	20.8
479 + 532 nm	$2.5 \times 10^{13} + 2.5 \times 10^{13}$	8.9×10^{13}	34.2

Table 2. Photon flux in 50 nm wavelength blocks for the monochromatic and polychromatic light stimuli used in this and our previous study.

	Photon flux (photons/cm^2/sec) for 50 nm wavelength blocks					
Light	**400 – 450**	**451 – 500**	**501 – 550**	**551 – 600**	**601 – 650**	**651 – 700**
437 nm	4.2×10^{13}					
479 nm		2.5×10^{13}				
532 nm			6.4×10^{13}			
4000 K	8.9×10^{12}	1.3×10^{13}	2.8×10^{13}	1.6×10^{13}	3.2×10^{13}	4.1×10^{12}
17000 K	9.1×10^{12}	1.4×10^{13}	1.6×10^{13}	6.4×10^{12}	7.4×10^{12}	
High pressure mercury**	1.6×10^{13}	9.6×10^{12}	1.5×10^{13}	2.6×10^{13}	7.0×10^{12}	1.1×10^{12}

**Revell and Skene, 2007

Analysis and statistics

Melatonin: Percentage melatonin suppression was calculated as the change in melatonin levels from 0 to 30 min as a percentage of the levels at 0 min.

Mood and alertness: The scores at each time point (-15, 0, 15, 30 and 45 min) were individually normalised to the score at 0 min for each light condition.

PVT: Tests were performed at three time points: 'pre', 'during' and 'post' light. Six measurements were extracted for analysis: mean reaction time (RT), median RT, mean reciprocal reaction time (RRT), number of lapses > 400 ms, mean slowest RT, mean slowest RRT.

Not all participants completed all light conditions: due to technical issues with the lights and problems with blood sampling one participant did not complete the 437 nm light condition, two participants did not complete the 17000 K light condition and two participants did not complete the 4000 K light condition. Thus, a total of seven participants completed all eight light conditions. Adequate melatonin profiles for analysis were obtained from 10 of the 12 participants.

All statistical analysis was carried out using Proc GLM repeated measures ANOVA analysis in the SAS program (SAS Institute Inc., Cary, NC, USA; Version 9.1). This analysis accounted for any missing values in the data sets. All analyses initially assessed whether there were any sequencing effects i.e. whether the study night significantly influenced the response. Only outcome variables exhibiting no sequencing effects were considered for further analysis. For the five 'melanopsin photon-matched' conditions, one-way repeated measures ANOVA analysis (factor: 'light condition') was conducted for the melatonin outcome variable and two-way repeated measures ANOVA analysis (factors: 'light condition' and 'time') was conducted for the PVT and normalised mood and alertness outcome variables. As the no-light conditions occurred on fixed nights they were not included in the initial analysis. However, for any parameters from the PVT and mood and alertness data that did not exhibit sequencing effects the ANOVA analysis was repeated to include a no-light condition (night 6). For the combined monochromatic light conditions two-way repeated measures ANOVA analysis (factors: 'light condition' and '479 nm presence') was conducted for the melatonin outcome variable and three-way repeated measures ANOVA analysis (factors: 'light condition', '479 nm presence' and 'time') was conducted for the PVT and normalised mood and alertness outcome variables to compare

437, 479 and 532 nm light with and without simultaneous presentation of 479 nm light. For all analyses significant effects were followed up with post-hoc tests utilising least squares estimates of marginal means.

RESULTS

The ability of eight light conditions, matched for their predicted efficacy to stimulate melanopsin but differing in their total photon density, to influence nocturnal non-visual responses was assessed.

Monochromatic and polychromatic lights matched for predicted melanopsin efficacy

A significant effect of light condition on the degree of nocturnal melatonin suppression was observed ($F_{(4, 31)} = 5.98$, $p = 0.0011$). Post-hoc comparisons revealed that 437, 479 and 532 nm light all induced significantly greater melatonin suppression than both 17000 K and 4000 K light ($p < 0.03$) (Figure 2). The degree of melatonin suppression did not appear to relate to the total photon content of the light stimulus; the lowest melatonin suppression was observed with the 4000 K light condition which had the highest total photon flux (1.0×10^{14} photons/cm^2/sec) and the greatest melatonin suppression was observed with the 437 nm light condition (4.2×10^{13} photons/cm^2/sec).

There was a significant effect of time ($F_{(4, 236)} = 4.84$, $p < 0.001$) and light condition ($F_{(4, 236)} = 3.51$, $p < 0.01$), but no significant light*time interaction, on subjective alertness. The level of alertness was significantly lower under the 479 nm light condition compared to the 437 nm, 532 nm, 4000 K and 17000 K conditions ($p < 0.01$). When the analysis was repeated to include a no-light condition the significant effects of time ($F_{(4, 299)} = 4.33$, $p < 0.01$) and light condition ($F_{(5, 299)} = 7.87$, $p < 0.0001$) remained; both the no-light and 479 nm light conditions exhibited significantly lower alertness levels than then remaining light conditions ($p < 0.02$); alertness under 479 nm light did not differ significantly from that under no-light (Figure 3). Of the remaining subjective scales, two (KSS and cheerfulness) exhibited sequencing effects and two (calmness and depression) did not vary significantly with light condition or time. Of the six PVT parameters measured, four of them exhibited sequencing effects and were not considered further. The remaining two parameters (median RT and mean SRT) exhibited no significant time or light condition effects.

Monochromatic lights presented alone and simultaneously with 479 nm

No significant effect of light condition, presence of 479 nm or light*479 nm presence interaction on melatonin suppression was observed (Figure 4). Thus, for the 479 nm light condition, doubling the irradiance did not significantly alter the melatonin suppression response. The PVT and subjective mood and alertness outcome variables exhibited either sequencing effects or no significant effects, with the exception of the KSS which exhibited a significant light*479 nm presence interaction.

DISCUSSION

The study aimed to determine whether the melanopsin photosensitivity function (λ_{max} 480 nm) could predict the magnitude of non-visual responses to nocturnal light. Thus, three monochromatic lights, administered singly and in combination with 479 nm, and two polychromatic lights of differing colour temperatures (4000 K and 17000 K) matched, for predicted melanopsin efficacy, were tested. If melanopsin were the sole contributor to nocturnal non-visual light responses it was hypothesised that lights matched for melanopsin efficacy would produce the same magnitude responses.

Indeed, for the monochromatic lights tested, no significant differences in the degree of melatonin suppression were observed amongst or between the single or the double intensity conditions. The lack of a statistically significant irradiance effect for the 479 nm light conditions (single vs double) may reflect limited resolution of the melatonin response. However, our data are in agreement with the previously constructed irradiance response curves (IRC) for melatonin suppression (Thapan et al., 2001) which did not demonstrate any significant increase in suppression between photon densities similar to those used in the current study.

The monochromatic light wavelengths were chosen to match the S-cone (437 nm) and M-cone (532 nm) peak sensitivities to try to determine any influence of these photopigments in comparison to melanopsin alone (479 nm). The lack of significant differences in melatonin suppression between the 437, 479 and 532 light conditions suggests either that the melanopsin photosensitivity function (λ_{max} 480 nm) is predictive of response magnitude or that the melatonin suppression response does not have the sensitivity to detect different visual photopigment contributions. The irradiances used in the current study were within the melanopsin operating range (> 10^{13} photons/cm^2/sec) and thus, if melanopsin is the

predominant photopigment driving the response then it is possible that any additional influence of cones was not able to be detected. Similar findings have been observed in mice whereby the magnitude of the pupil constriction response is accounted for solely by melanopsin once the light irradiance is within the melanopsin operating range and melanopsin is activated (Lucas et al., 2003) under a 'winner takes all' arrangement (Lall et al., 2010). However, previous human studies using long duration nocturnal light stimuli of similar photon densities to those used here (2.8×10^{13} photons/cm^2/sec) have implicated a role for cones in the melatonin suppression response (Lockley et al., 2003). Very recently, dose response curves to 460 nm and 555 nm light for the melatonin suppression and phase delaying responses have been constructed in humans (Gooley et al., 2010). Their findings demonstrated that cones can drive an initial melatonin suppression response but this is not sustained. Further studies are required to fully understand the role and response dynamics of cones in human non-visual light responses.

Even though photon matched for their ability to stimulate melanopsin both the 4000 K and 17000 K lights exhibited significantly lower melatonin suppression compared to the monochromatic light conditions. The fact that a simple photosensitivity function cannot solely determine the efficiency of a polychromatic light stimulus to suppress melatonin, however, may not be so surprising when the assumptions made to produce this prediction are considered. Firstly, it is assumed that the calculated effective photon density is absorbed solely and entirely by melanopsin to generate a measurable physiological response. However, for a polychromatic light stimulus the photons across the visible light range are available for absorption not only by melanopsin but also by the rods and cones. In addition, it is assumed that the photons are absorbed at a constant rate which may not be the case. Finally, the critical integration period over which melanopsin absorbs photons and drives specific responses is not known. In a dynamic system there could be many factors at play and more information is needed before a full model can be generated that will allow the response to broadband lighting to be predicted.

Our previous work also demonstrated that the response to polychromatic light cannot be predicted by a photosensitivity function with λ_{max} 480 nm (Revell and Skene, 2007) but in that study the ultra high-pressure mercury lamp produced greater melatonin suppression than was predicted. The discrepancy between our two studies may relate to the different spectra of the polychromatic light sources used. A comparison of the photon flux in 50 nm wavelength

blocks for the polychromatic lights (Table 2) revealed the greatest difference between the ultra high-pressure mercury lamp and the 4000 K and 17000 K lights used in the current study occurred in the very short wavelength block (400 – 450 nm) and the medium wavelength block (551 – 600 nm). The mercury lamp contained almost twofold the number of photons between 400 and 450 nm than the 4000 K or 17000 K lights. In addition, in the 551 – 600 nm block the mercury lamp contained four times the number of photons than the 17000 K light and 60% more photons than the 4000 K light. The increased photon flux in the 551 – 600 nm range for the high pressure mercury lamp could enhance the melatonin suppression via stimulation of the M- and L- cones or via the photoreversal function of melanopsin as both *in vitro* and *in vivo* data have implicated 540 – 590 nm as being the optimal wavelength range for inducing the photoreversal of melanopsin (Melyan et al., 2005; Mure et al., 2007; Mure et al., 2009).

The increased number of short wavelength photons in the high pressure mercury lamp combined with the fact that the greatest melatonin suppression in the current study occurred with the 437 nm light, suggests that the short wavelength content of the light stimulus may be a strong factor in determining the response magnitude. This postulated sensitivity to short wavelength light could reflect either a role for the S-cones or that the photosensitivity function of human melanopsin is maximally sensitive to shorter wavelengths than the rodent data suggest. Involvement of S-cones in human non-visual responses has previously been suggested (Thapan et al., 2001; Revell et al., 2006). We demonstrated that the subjective alerting response to morning light was sensitive to very short wavelengths (420 nm) (Revell et al., 2006) and the high sensitivity of the melatonin suppression response to 424 nm light was suggested to reflect S-cone involvement (Thapan et al., 2001). In addition, anatomical work in non-human primates has demonstrated that the S-cones exhibit a rare OFF response to the melanopsin-RGCs whereby they fire when the lights are turned off (Dacey et al., 2005); the significance of this OFF signal, however, remains unclear. Alternatively it is possible that the spectral sensitivity of human melanopsin may be different to rodent melanopsin. The published melatonin suppression action spectra showed a $\lambda_{max} \sim 460$ nm and the maximal melatonin suppression was observed at the 440 and 456 nm point, respectively (Brainard et al., 2001; Thapan et al., 2001) on non lens-corrected data. *In vitro* expression of human melanopsin demonstrated an enhanced sensitivity to 420 nm compared to 480 nm (Melyan et al., 2005) which could not be reconciled with the animal data. It was

thus postulated that this deviation may reflect an influence of the cell type used for the melanopsin expression work. However, it is entirely possible that human melanopsin has a different spectral sensitivity to rodent melanopsin considering the nocturnal/diurnal differences between these species, the fact that different types of visual photopigments are expressed and that light can induce opposite effects e.g. sleep versus wake.

The alerting effect of light at night observed in the current study supports previous work with both monochromatic and polychromatic light (Cajochen et al., 2000; Cajochen et al., 2005; Lockley et al., 2006; Revell et al., 2006; Sletten et al., 2009). However, in contrast to the melatonin suppression data the alertness levels were significantly lower under the 479 nm light condition compared to the 437 nm, 532 nm, 4000 K and 17 000 K light conditions. Although this finding is at odds with previous studies demonstrating an enhanced alerting effect of 460 nm over 555 nm light (Lockley et al., 2006; Cajochen et al., 2005), differences in the study protocols could explain these discrepancies. Firstly, the participants were awake for several hours before the light exposure (Lockley et al., 2006; Cajochen et al., 2005), compared to 30 min in our protocol, secondly, the lights were administered at different circadian phases: a) fixed clock time of 21:30 – 23:30 h (Cajochen et al., 2005), b) beginning ~6.75 h before the core body temperature minimum (Lockley et al., 2006) and thirdly, the light exposures were for extended durations (2 or 6.5 h) compared to 30 min in our protocol. When a 6.5 h light stimulus was used (Lockley et al., 2006) after one hour of light exposure the alertness levels under 460 nm and 555 nm light do not appear to be significantly different suggesting that spectral differences to nocturnal light may only be observed under extended duration stimuli. In the current study, the alerting efficacy of light appeared to relate to the total photon content of the stimulus as the effective light conditions had the highest irradiances. Furthermore, the observed alerting effects of 437 nm and 532 nm light may implicate a role for cones in the alerting response to short duration light. A contribution of cones to non-visual responses involving alerting/sleep centres over the first 30 min of a light stimulus has previously been demonstrated in mice (Altimus et al., 2008).

In conclusion, the findings support our previous work (Revell and Skene, 2007) and demonstrate that the melanopsin photosensitivity function (λ_{max} 480 nm) cannot predict non-visual responses to polychromatic light. Obviously, the current dataset has only assessed one 'melanopsin-stimulating' photon density and complete fluence response curves to polychromatic light need to be constructed and compared to monochromatic light fluence

response curves to fully understand non-visual light responses. Predicting non-visual responses to polychromatic light will likely be a complex procedure due to possible multiple photopigment involvement, the potential melanopsin photoreversal function, and the physiology and dynamics of both photon detection and signal processing. Further work is required to determine these interactions and dynamics.

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Figure Legends

Figure1. Spectral power density of a) monochromatic light conditions (λ_{max} 437, 479 and 532 nm) and b) polychromatic light conditions (colour temperature: — 4000 K and ----17000 K) as measured *in situ* with a calibrated spectrometer.

Figure 2. Percentage melatonin suppression (mean ± SEM) following a 30 min nocturnal light stimuli (2.5 x 10^{13} 'melanopsin stimulating' photons/cm^2/sec) of monochromatic (437, 479 and 532 nm) or polychromatic light (4000 K and 17000 K) matched for predicted melanopsin stimulation. * $p < 0.05$ compared to 437, 479 and 532 nm light.

Figure 3. Normalised subjective alertness (mean ± SEM) from 15 min before until 45 min after the start of a 30 min nocturnal light stimulus: ● no-light, ▲437 nm, ○ 479 nm, ■ 532 nm, □ 17000 K and △4000 K.

Figure 4. Percentage melatonin suppression (mean ± SEM) following a 30 min nocturnal light stimulus of monochromatic 437, 479 and 532 nm light matched for predicted melanopsin stimulation: with (□ 5.0 x 10^{13} photons/cm^2/sec) and without (■ 2.5 x 10^{13} photons/cm^2/sec) simultaneous administration of 479 nm monochromatic light.

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