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ORIGINAL ARTICLE

Demonstration of additivity failure in human circadian phototransduction

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Abstract

OBJECTIVES: Published data, both on nocturnal melatonin suppression in humans and on widely accepted retinal structure and function, suggest that spectral opponency plays a role in human circadian phototransduction. We directly test subadditivity, implied by spectral opponency, in human circadian phototransduction in response to nearly monochromatic and to polychromatic light.
METHODS: Adult male human subjects were exposed for 60 minutes to two intensities each of two lighting conditions, during nighttime experimental sessions. One condition consisted of light from mercury vapor lamps (450 and 1050 lx), and one condition consisted of light from these lamps filtered such that only the spectral line from this lamp at 436 nm was presented to subjects (7.5 and 15 lx).
RESULTS: Melatonin suppression from the filtered illumination at 436 nm alone was greater than mercury lamp illumination (containing energy at 436 nm in addition to other wavelengths), even when the sources exposed subjects' retinae to equal amounts of irradiance at 436 nm.
CONCLUSION: This direct test of subadditivity, together with evidence from neuroanatomy, supports the inference that spectral opponency is a fundamental characteristic of human circadian phototransduction.

Abbreviations

CV	– coefficient of variation
Hg	– mercury vapor
RGC	– retinal ganglion cell
RIA	– radioimmunoassay
SCN	– suprachiasmatic nucleus
SPD	– spectral power distribution

Introduction

Regulation of nocturnal melatonin by light in humans was discovered more than 25 years ago [11]. Subsequently, it was demonstrated that regulation of the mammalian circadian system, of which melatonin production is a marker, occurs

strictly through the ocular photoreceptors [6,12,15]. Since these milestone studies, several attempts have been made to identify the mechanism for circadian phototransduction in humans. Among the most comprehensive recent studies along these lines are those of Brainard et al. [3] and Thapan et al. [24], who each developed action spectra for human nocturnal melatonin suppression. Both groups suggested that their respective results closely matched a single opsin photopigment with peak sensitivity around 460 nm, in the short-wavelength region of the visible spectrum. Rea et al. [18,19], Wright and Lack [27], Lockley et al. [13] and Fucci et al. [9] also demonstrated short-wavelength sensitivity for melatonin suppression, while Wright and Lack [27], Warman et al. [25], Lockley et al. [13], Wright et al. [28] and Revell et al. [20] showed that circadian phase shifting in humans is also dominated by short-wavelength sensitivity. It is unlikely, however, that a single opsin photopigment is responsible for circadian phototransduction in mammals, including humans, based on recent data. Earlier studies showed that genetically-manipulated mice lacking rod and cone photoreceptors retain circadian responses to light [14]. In 2002, Berson and colleagues, (see Berson [2] for a review) using electrophysiological responses, identified photosensitive RGCs containing melanopsin with spectral sensitivity peaking near 480 nm in rat. Similar cells with the similar spectral sensitivity of electrophysiological responses have been identified in macaque [5]. Ruby et al. [21] and Panda et al. [16] showed that melanopsin-deficient mice could still be entrained to light/dark cycles and was still able to show phase-shifting response to bright white light and monochromatic light at 480 nm respectively, although these responses were attenuated by approximately 40% and 45%, suggesting that although melanopsin is not essential for the SCN to receive light stimulus, it contributes significantly to the magnitude of the response. More recently, however, genetically-manipulated mice lacking melanopsin coupled with a rod-cone system that is unable to signal light have no circadian responses to light [10,17]. Bullough et al. [4] demonstrated that multiple photoreceptive inputs were needed to best explain circadian locomotor activity phase shifts in mice with intact retinae.

These data, as well as findings of rod-cone interactions in neurons projecting to the suprachiasmatic nucleus in rats [1] and multiple photoreceptor inputs to melanopsin-containing RGCs in macaque [5], lead to the inference that more than one photoreceptor is responsible for mammalian circadian phototransduction. Indeed, Rea et al. [19] observed that both of the recent human action spectra [3,24] could be explained by combining sensitivity from multiple photoreceptors.

Figueiro et al. [8] measured melatonin suppression in humans and suggested that an S-cone spectral opponent mechanism contributes to the spectral sensitivity of the SCN, directly challenging assumptions of additivity for human circadian phototransduction. Specifically, they argued that an S-ON, blue minus yellow (b–y), spectral opponent mechanism [29] provided input to the melanopsin-containing RGCs. Dacey et al. [5] also showed a plausible link between melanopsin-containing RGCs and an S-cone opponent mechanism. They recorded electrical signals in the lateral geniculate nucleus (LGN) of macaque, a species with a similar visual system as humans. Unlike the evidence for an S-ON response from Figueiro et al. using nocturnal melatonin suppression, Dacey et al. provide electrophysiological evidence for an S-OFF (y–b) spectrally opponent response in the LGN. The LGN is an essential neural relay center to the visual cortex, but there is no evidence to suggest that the LGN plays an important role in circadian regulation. Thus, although both studies provide evidence for an interaction between S-cone spectral opponent mechanisms and melanopsin-containing RGCs, it would seem from these two preliminary studies that the S-cone input to the SCN is opposite in polarity from the S-cone input to the LGN.

One of the light sources used by Figueiro et al. [8], a clear Hg lamp, is particularly useful in assessing additivity because its SPD is essentially discrete with a significant emission line near 436 nm, close to the peak sensitivity for melatonin suppression identified by Brainard et al. [3] and Thapan et al. [24]. The Hg lamp SPD also contains emission lines near 546 and 578 nm, as well as a small emission line near 406 nm. With strategic filtering, this lamp provides an opportunity to assess additivity directly. Spectral opponency [8] predicts that exposure to the short-wavelength (436 nm) component from Hg illumination will result in greater melatonin suppression than exposure to that same component in combination with the 546 and 578 nm components, even though the irradiance from the entire SPD is much higher than that from the 436-nm component alone. This paper describes a test of additivity utilizing this approach using Hg lamp illumination.

Material & Methods

Four males aged from 34 to 53 years participated in the study, which occurred during two nights. Experimental sessions lasted 5 h (from 23:00 to 04:00 h). Approval from Rensselaer's Institute Review Board was obtained before the study.

Four experimental conditions (described below) were presented in 0.6 × 0.6 × 0.6 m light boxes painted matte white inside and containing square 0.45 × 0.45 m apertures and chinrests in the front so that subjects viewed the interiors. The boxes were illuminated indirectly by 175 W Hg lamps (General Electric, HR175A39). For two of the conditions the Hg illumination was diffused with acrylic diffusers such that one condition produced an illuminance of 450 lx at subjects' corneas and the other produced 1050 lx at subjects' corneas. For the other two conditions, the diffusers were fitted with a colored filter (Lee, No. 120) that absorbed the 546- and 578-nm emission lines and passed the short-wavelength lines. Under one filtered condition, the resulting corneal

Table 1: Mean pupil area for each experimental condition, and resulting irradiance values (irradiance × pupil area) at 436 nm for each condition.

Experimental condition	Pupil area (mm^2)	Retinal irradiance at 436 nm, lens- and pupil-corrected [$(W/m^2)\cdot mm^2$]	Relative retinal irradiance at 436 nm (%)
436 nm (low)	9.6	2.81	52%
436 nm (high)	6.9	4.09	75%
Hg lamp (low)	8.0	2.74	51%
Hg lamp (high)	6.9	5.42	100%

illuminance was 7.5 lx and under the other, the corneal illuminance was 15 lx. All of these conditions were selected to provide irradiances likely to fall within the dynamic range of the melatonin suppression response, based on previous studies [7].

On each night, blood samples (3 ml) were drawn from subjects every 15 min. from 00:30 to 04:00. From 01:00 to 02:00 and from 03:00 to 04:00, the subjects were exposed to light. At all other times, subjects remained seated wearing dark sunglasses in the dimly illuminated area (less than 1 lx at the eye). The two samples taken before exposure to each light condition (between 00:30 and 01:00 and between 02:30 and 03:00) served as the reference melatonin levels in darkness, and the last two samples taken during each light exposure period served as the experimental conditions. Samples were immediately spun in a centrifuge at 3200 rpm for 10 min. and the plasma was frozen at -85ºC. Frozen samples were sent to an independent laboratory (Neuroscience Inc., Osceola, WI) for melatonin^{direct} I-125 RIA. The detection limit of the assay was 1.5 pg/ml. The intra-assay CVs were 12.1% at 16.5 pg/ml, 5.7% at 68.7 pg/ml, and 9.8% at 162.7 pg/ml. The inter-assay CVs were 13.2% at 17.3 pg/ml, 8.4% at 69 pg/ml, and 9.2% at 164.7 pg/ml. Each subject experienced the two Hg conditions on one night and the two 436-nm conditions on a different night. Two subjects received the Hg high condition from 01:00 to 02:00 and two received the Hg high condition from 03:00 to 04:00. The order of the 436-nm conditions were similarly reversed for the two pairs of subjects, so that each set of measurements contained equal proportions of data corresponding to the 01:00 to 02:00 period as to the 03:00 to 04:00 period.

Because each subject's pupil area affects the actual retinal exposure under each condition, pupil size mea-

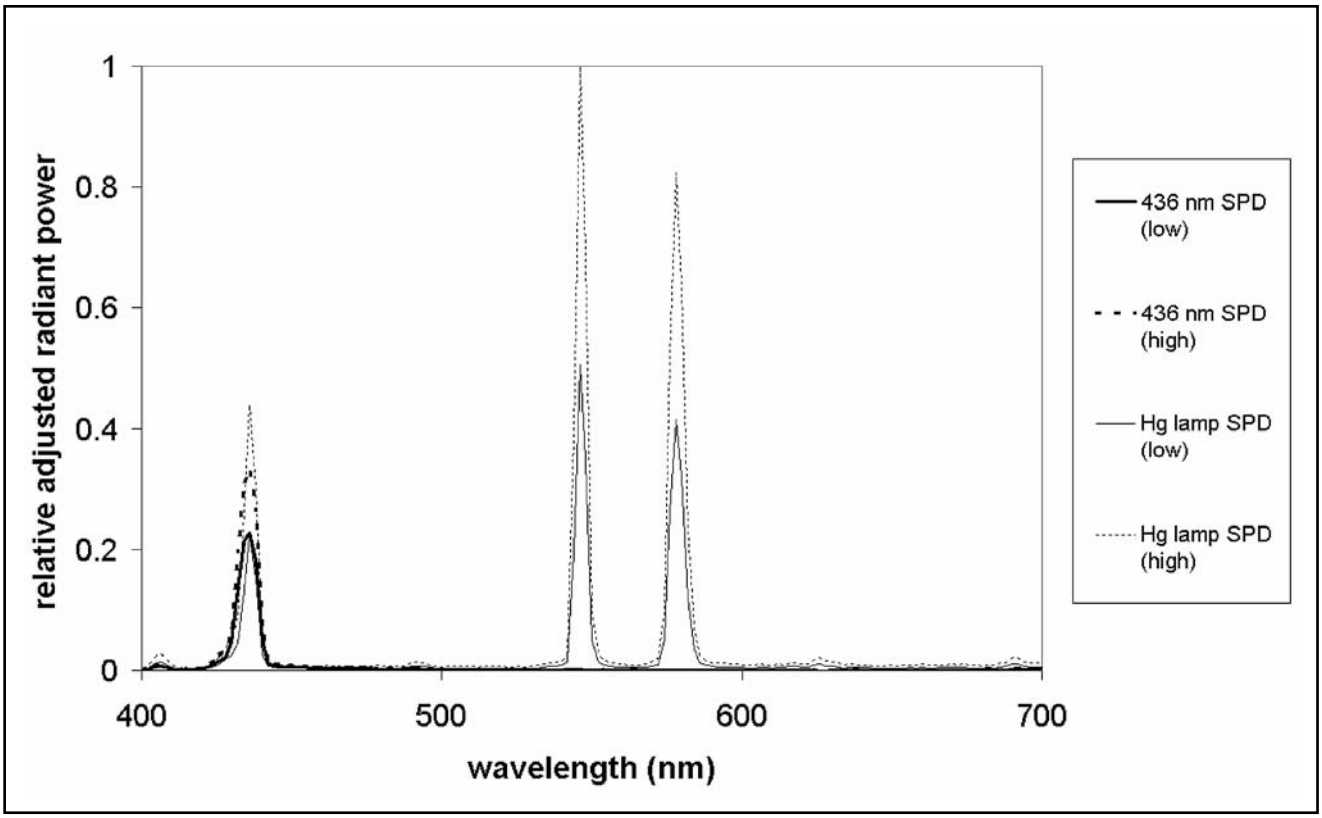

Figure 1. Relative SPDs for the four experimental conditions, scaled for pupil area and lens transmission.

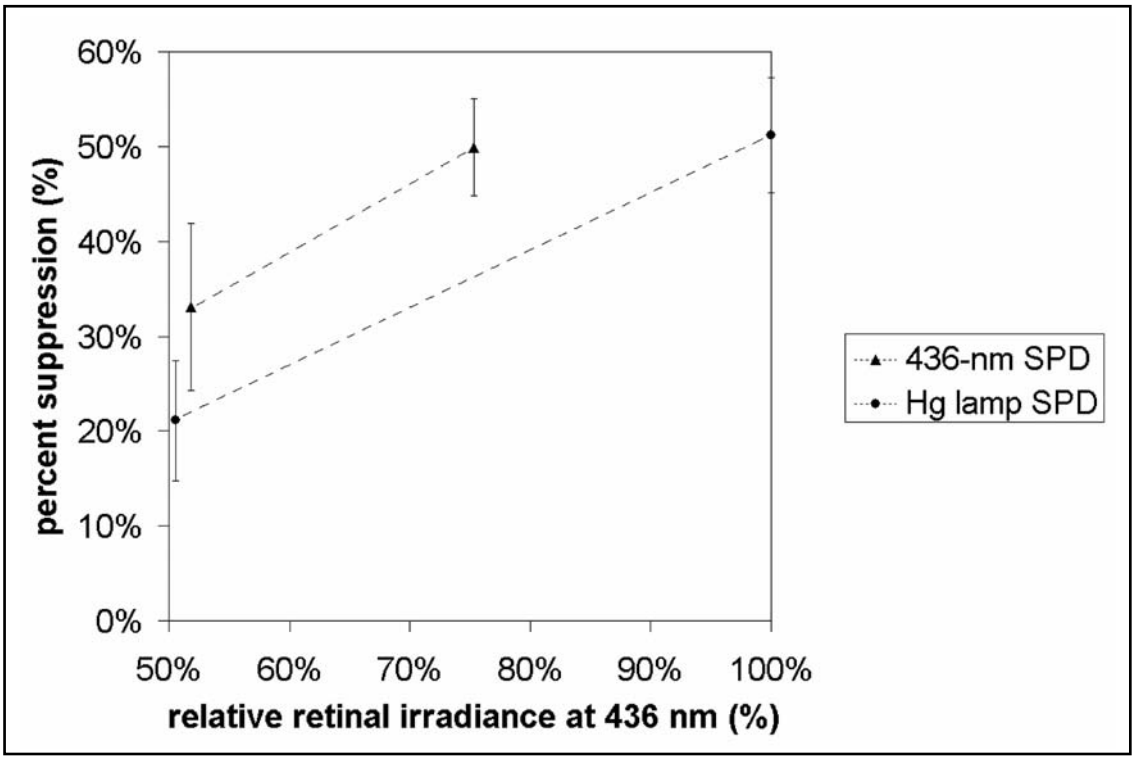

Figure 2. Mean melatonin suppression (±s.e.m.), plotted as a function of the relative radiant power at 436 nm exposed to the retina (lens- and pupil-corrected) for each experimental condition.

surements were also made prior to the experimental sessions using infrared videography in the same apparatus and under each of the lighting conditions used in the study. In this way, the irradiances provided by each condition were adjusted to account for differences in pupil area and by changes in lens spectral transmission across the radius of the human lens, as described by Weale [26]. *Figure 1* shows the relative SPDs for the four experimental conditions, adjusted for pupil area and lens spectral transmission. *Table 1* lists for every condition the mean pupil area (mm^2), the absolute lens- and pupil-corrected retinal irradiance at 436 nm (W/m^2 × pupil area in mm^2), and the relative retinal irradiance at 436 nm (%).

Results

The mean melatonin suppression values (± s.e.m.) for each of the four experimental conditions are plotted in *Figure 2*, as a function of the relative 436-nm retinal irradiance values for each condition. The data for the Hg lamp conditions and for the 436-nm conditions fall along two nearly parallel lines, suggesting that the melatonin suppression values correspond to the nearly linear portion of the sigmoidal dose-response function for this response [30], and not in the saturated portion of such a dose-response function. This inference is further supported by findings in other studies [3,24] showing a maximum suppression value of about 70% to 75% (compared to about 50% in this study) for similar durations of nighttime light exposure. For each SPD, one-tailed student's t-tests revealed statistically reliable ($p<0.05$) effects of light level, with the high levels resulting in greater melatonin suppression. Using the retinal irradiance value at 436 nm for each condition (listed in *Table 1*) as a covariate in a repeated-measures analysis of variance [22], there was also a statistically reliable ($p<0.05$) effect of SPD, with the 436-nm illumination resulting in greater melatonin suppression than the Hg lamp illumination.

Discussion

The data in *Figure 2* provide direct evidence for additivity failure in human nocturnal melatonin suppression, consistent with the data of Figueiro et al. [8], which supported subadditivity through spectral opponency. For example, the lower irradiance, 436-nm SPD (low-436) and the lower irradiance, Hg lamp SPD (low-Hg), once corrected for differences in pupil area and lens transmission [26], had very similar retinal irradiance values at 436 nm. As seen in *Figure 1*, the low Hg lamp SPD also had significant power at 546 and 578 nm. Assuming additivity, and even assuming that spectral sensitivity for melatonin suppression at 546 and 578 nm is negligible [3,24], the low-Hg should have resulted in at least the same melatonin suppression as the low-436. In fact, the analysis of variance using the 436-nm retinal irradiance value as a covariate [22] showed that melatonin suppression for the low-Hg was statistically significantly lower than for the low-436, implying that the radiant power at the longer wavelengths in the Hg

lamp SPD actually reduced the neural response, and that the human circadian system has a spectrally opponent sensitivity function, consistent with the report by Figueiro et al. [8].

It is now well established that a novel photosensitive melanopsin-containing RGCs as well as traditional photoreceptors contribute to phototransduction by the mammalian circadian system [10,16], although the exact neural circuits in humans that combine inputs from the traditional photoreceptors with the novel ganglion cell have not been analyzed nor identified in the literature. Given the known retinal neuroanatomy in primate [5], it is reasonable to suppose that spectral opponency, formed prior to the ganglion cell layer contributes to phototransduction by the human circadian system. What seems to be clear, however, is that considerations of spectral opponency will play a role in future assessments of the impact of architectural lighting on the circadian system [23].

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