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## Human melatonin action spectrum: relevance to circadian phototransduction

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**ABSTRACT:**

The broad goal of this research is to identify the human photoreceptor system that mediates circadian physiology, neuroendocrine regulation and the clinical benefits of light therapy. Recently, there has been an upheaval in the understanding of circadian photoreception in mammals. Specifically, it has been shown that the rodent retinal ganglion cells which project to the suprachiasmatic nuclei are directly photosensitive and have a peak sensitivity in the short-wavelength visible spectrum (Berson et al., Science 2002; Hattar et al., Science 2002). Furthermore, two photopigments in the mammalian ganglion cell layer, melanopsin and cryptochrome, have been identified as candidates for circadian phototransduction (Provencio et al., PNAS 1998; Miyamoto and Sancar, PNAS 1998). The specific aim of the following study was to elucidate the ocular photoreceptor system responsible for regulating the human pineal gland by establishing an action spectrum for light-induced melatonin suppression. Healthy male and female subjects participated in over 650 nighttime melatonin suppression tests with monochromatic wavelengths from 420 nm to 600 nm. Blood samples collected before and after light exposures were quantified for melatonin. The data were fit to univariant, sigmoidal fluence-response curves ( $R^2 = 0.81 - 0.95$ ). The action spectrum constructed from these curves fit an opsin template ( $R^2 = 0.91$ ). The results suggest that, in humans, a single short-wavelength sensitive photopigment is primarily responsible for melatonin suppression and its peak absorbance appears to be distinct from that of rod and cone cell photopigments for vision (Brainard et al., J. Neuroscience 2001). Independently, another laboratory has published data that similarly suggests an opsin photopigment contributes to human melatonin suppression (Thapan et al., J. Physiology 2001). These action spectra indicate a novel retinaldehyde-based photopigment mediates light input to the human retinohypothalamic tract: Support: NIH RO1NS36590 and the National Space Biomedical Research Institute under NASA Cooperative Agreement NCC 9-58.

**Keywords:** *action spectrum, melatonin, photoreceptor, circadian*

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## Human Circadian Photoreception: Short Wavelength Sensitivity

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### ABSTRACT:

The ocular photoreceptor(s) mediating non-image forming, circadian responses to light (eg circadian entrainment) have not yet been characterised in humans. Recent research by ourselves<sup>1</sup> and others<sup>2</sup> has shown that a novel non-rod, non-cone photoreceptor may be involved. Using the ability of light at night to suppress plasma melatonin, the spectral sensitivity of this response was investigated. The half-maximal responses, obtained from 6 individual irradiance response curves, were corrected for lens filtering and used to construct an action spectrum. The findings showed that light-induced melatonin suppression in humans is sensitive to short wavelength light (420-480 nm), a response very different to the classical scotopic and photopic visual systems. Although the action spectrum best fitted a rhodopsin template ( $\lambda_{\max}$  459 nm) suggesting a novel opsin to be the most likely candidate, the high sensitivity observed at the shortest wavelength (424 nm) left some doubt. A shorter wavelength ( $\lambda_{\max}$  415 nm) was thus investigated at 5 different irradiances (1-40 microW/cm<sup>2</sup>, n=6/irradiance) in the same protocol. Correcting for lens transmission, 415 nm light was 3-fold less effective than 424 nm light at suppressing melatonin. The resultant action spectrum still best fitted a rhodopsin template with  $\lambda_{\max}$  459 nm, confirming our original data<sup>1</sup>. Whether other nonvisual light responses (eg circadian phase resetting, enhanced alertness) also show short wavelength sensitivity is currently being studied. Our preliminary findings<sup>3,4</sup> suggest a similar short wavelength sensitivity of these non-image forming light responses. 1. Thapan, K. et al., An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J. Physiol.* (2001) 535, 261-7. 2. Brainard, G.C. et al., Action spectrum for melatonin regulation in humans: Evidence for a novel circadian photoreceptor. *J Neuroscience* (2001) 21, 6405-12. 3. Hoppen, K. et al., The effects of spectral composition of light on alertness, performance and melatonin. SLTBR Meeting, Stockholm, 2001. 4. Warman, V. et al., Phase shifting the human melatonin rhythm with short wavelength light. SRBR Meeting, Florida, 2002.

**Keywords:** *action spectrum, wavelength, circadian, human*

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## Phototransduction in the Human Circadian System

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**ABSTRACT:**

Human circadian rhythms are entrained to the environmental 24-h light-dark cycle exclusively by ocular photoreception. The photoreceptor mediating entrainment is unidentified although confirmation of light-induced pineal melatonin suppression in totally blind humans and the spectral sensitivity of the suppression response in normal, sighted subjects (peak region of sensitivity 446-477 nm) suggests the involvement of a non-rod, non-cone ocular photoreceptor in retina-RHT-SCN-pineal signaling. The aim of our study was to assess the magnitude of the circadian phase-shifting response and light-induced melatonin suppression following ocular exposure to equal photon densities of monochromatic light at 460 nm and 555 nm, and therefore to indirectly assess the spectral properties of the photoreceptor(s) mediating circadian responses. We studied 12 subjects (4F; aged 21-28 yrs) in an environment free of time cues for 9 days. After a 3-day baseline (150 lux), subjects underwent a 50h constant routine (CR) in <2 lux to assess initial circadian phase, and a second 30h CR to assess final phase after experimental light exposure. Subjects were exposed to one 6.5h episode of monochromatic light timed to induce a phase-delay. Monochromatic light was generated via a grating monochromator connected to a 1200W xenon arc lamp. Subjects gazed into a modified Ganzfeld sphere and remained seated during and for 90 minutes prior to exposure. Their pupils were dilated (0.5% Cyclopentolate HCl) 15 minutes before exposure. Subjects were randomized to either the 460nm or 555nm exposure (10nm half-peak bandwidth) of equal photon density ( $28 \times 10^{12}$  photons/cm<sup>2</sup>/sec). Plasma was sampled every 20-30 minutes and rectal core body temperature measured continuously. We hypothesize that a significantly greater phase-shift of plasma melatonin and core body temperature rhythms will be observed following exposure to an equal photon density of monochromatic light at 460 nm compared to 555 nm. We further predict that the suppression of melatonin in the first 90 minutes of exposure will be greatest following exposure to 460 nm light. The results of this study will indicate whether the photoreceptor system mediating circadian responses is primarily photopic or non-photopic. This work was supported by the NIH (RO1NS36590).

**Keywords:** *circadian, phase-shift, melatonin, monochromatic light*

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## How many photopigments are involved in circadian photoreception ?

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**ABSTRACT:**

In humans, as in other mammals, light is the main synchronizing factor of the circadian clock. Although the retinal photoreceptor types involved in transmitting light information to the SCN are unknown the spectral properties can be described by measuring the sensitivity of the response to light at different wavelengths. We assessed the sensitivity of the circadian system in humans by measuring light induced plasma melatonin (MEL) suppression at 9 wavelengths in 5 subjects. Plasma MEL levels were measured using RIA in samples collected several hours before, during, and several hours after a 1-hr nocturnal light stimulation. MEL suppression was calculated by comparing plasma MEL values after the light stimulation with levels collected at the same time during dark control sessions. We applied a model which fits the dose response curves for each wavelength, minimizing the residual sum of squares for all wavelengths in a single curve fitting procedure. The results indicate a peak sensitivity around 480-500 nm, although the width of the action spectrum suggests involvement of more than one photopigment. We then used a second model which further determines whether one or more photopigments are involved and which characterises the spectral and irradiance sensitivity of each photopigment. This model does not require an a priori assumption of the underlying number, wavelength, or threshold sensitivity of the photopigments. The same method was also used for analysing the photic responses of the circadian system in the mouse. The method predicts that at least 2 photopigments are involved in circadian photoreception in the mouse and human. Supported by: Biomed2 (BMH4CT972327, HFSP (RG95/68B))

**Keywords:** *photopigment, circadian, melatonin, human*

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## New insights into the diurnal regulation of the cone pathway

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ABSTRACT:

Of all mammalian organs the eye shows some of the most marked adaptations to time of day. In the human retina, second order processing of signals originating in cones takes significantly longer at night than during the day (Hankins et al., 1998) and this may represent an adaptation of the prevailing diurnal status. Long-term light exposure at night is capable of reversing this effect. Recently we have employed the cone ERG as a tool to examine the properties of the irradiance measurement pathway driving this reversal. Our findings indicate that this pathway: (A) integrates irradiance measures over time periods ranging from at least 15 to 180 min; (B) responds to relatively bright light, having a dynamic range almost entirely outside the sensitivity of rods; (C) acts on the cone pathway primarily through a local retinal mechanism; (D) detects light via an opsin: vitamin A photopigment ( $\lambda_{max} \sim 483\text{nm}$ ). We conclude that a photopigment with a spectral sensitivity profile quite different from those of the classical rod and cone opsins drives adaptations of the human primary cone visual pathway according to time of day. The data suggest a revision of models of the primary afferent cone pathway to include input from this novel irradiance measurement channel.

**Keywords:** *novel, photopigment, human*

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## Human Extraocular Circadian Phototransduction Revisited

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### ABSTRACT:

Four years ago, bright, narrow spectrum blue light exposure to the back of the knees was reported to reset the human circadian pacemaker(1). The report of extraocular photoreception in humans was contrary to the well-established finding that removal of the eyes leads to loss of circadian photoreception in mammals(2). We report here results from a replication study that examined bright light exposure to the back of the knees-using the same light source of the same light intensity, for the same duration of time as that employed in the original report. Twenty-two inpatient phase-resetting trials were carried out. Participants (28.63 ±9.4 years old) lived in the laboratory for 10 days and were scheduled to sleep for 8 h at their habitual bedtimes for 3 baseline days. This was followed by a 40-h constant routine (CR) protocol that was used to assess circadian phase. Following the CR, individuals were scheduled to an 8-h recovery sleep episode. On night 6 of the study, subjects were awakened to a 3-h intervention, with the mid-point of the intervention scheduled ~3 h before the temperature minimum, designed to produce a phase delay shift. Subjects were randomly assigned to one of three conditions: a) ocular exposure of ~9,500 lux and popliteal exposure of 0 lux; b) ocular exposure of 0 lux and popliteal exposure of up to 13,000 lux; c) ocular exposure of 0 lux and popliteal exposure of 0 lux. Since both conditions b and c were tested in total ambient darkness, a double blind procedure was used for the exposure of bright light versus darkness to the back of the knees. A second CR on days 8 and 9 was used to assess circadian phase after exposure to these interventions. Bright light exposure to the back of the knees failed to elicit a significant circadian phase delay shift. In contrast, we found that ocular exposure to bright light for the same duration of time at the same circadian phase induced a significant circadian phase-delay shift, compared to both the light behind the knee condition and the total darkness control condition. These results do not support circadian photoreception in response to bright light exposure to the back of the knees in humans. (1). S. S. Campbell and P. J. Murphy (1998) *Science* 279, 396-399; (2). R. G. Foster (1998) *Neuron* 20, 829-832.

**Keywords:** *Extraocular Photoreception, Circadian Rhythm, Phase Shift, Bright Light*

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## Photic resetting of the human circadian pacemaker

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ABSTRACT:

The human circadian pacemaker is exquisitely sensitive to light, which is its strongest environmental synchronizer. The phase resetting response to light is dependent upon the timing and intensity of the light exposure. As in other species, light exposure during the evening and early subjective night phase delays the human circadian pacemaker (as is required for westward travel), whereas light exposure during the late subjective night and morning phase advances the human circadian pacemaker (as is required for eastward travel). Unlike some organisms, humans do not exhibit a "dead zone" of photic insensitivity to the resetting effects of light. Exposure to episodes of bright light over three consecutive days can induce large (Type 0) phase shifts--resetting the endogenous pacemaker to any desired time, often via antecedent suppression of circadian amplitude. Weaker (Type 1) phase shifts can be elicited through exposure to light outside the critical region of strong resetting, to light of reduced intensity or to fewer cycles of light exposure. The human circadian pacemaker can be reset and be entrained by exposure to very dim light. A strongly non-linear dose response curve for the phase shifting effects of light has been described. Circadian photoreception--including both melatonin suppression and the induction of light-induced phase shifts--can be preserved notwithstanding the complete absence of conscious light perception in some blind individuals. Future research is required for quantifying the spectral sensitivity of the endogenous circadian pacemaker to light; quantifying the influence of the duration of light exposure on the magnitude of the circadian phase resetting response; and identifying the photoreceptor(s) necessary and sufficient for circadian phototransduction in humans.