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Short-wavelength enrichment of polychromatic light enhances human melatonin suppression potency

Abstract: The basic goal of this research is to determine the best combination of light wavelengths for use as a lighting countermeasure for circadian and sleep disruption during space exploration, as well as for individuals living on Earth. Action spectra employing monochromatic light and selected monochromatic wavelength comparisons have shown that short-wavelength visible light in the blue-appearing portion of the spectrum is most potent for neuroendocrine, circadian, and neurobehavioral regulation. The studies presented here tested the hypothesis that broad spectrum, polychromatic fluorescent light enriched in the short-wavelength portion of the visible spectrum is more potent for pineal melatonin suppression in healthy men and women. A total of 24 subjects were tested across three separate experiments. Each experiment used a within-subjects study design that tested eight volunteers to establish the full-range fluence–response relationship between corneal light irradiance and nocturnal plasma melatonin suppression. Each experiment tested one of the three types of fluorescent lamps that differed in their relative emission of light in the short-wavelength end of the visible spectrum between 400 and 500 nm. A hazard analysis, based on national and international eye safety criteria, determined that all light exposures used in this study were safe. Each fluence–response curve demonstrated that increasing corneal irradiances of light evoked progressively increasing suppression of nocturnal melatonin. Comparison of these fluence–response curves supports the hypothesis that polychromatic fluorescent light is more potent for melatonin regulation when enriched in the short-wavelength spectrum.

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Introduction

Light is a powerful stimulus for regulating human circadian, neuroendocrine and neurobehavioral responses [1–3]. Light also has the capacity to restore human health in clinical applications such as treating winter depression and selected sleep disorders. In addition, light therapy has been evaluated for healthy individuals who experience problems associated with intercontinental jet travel, shift work, and space flight [1–6].

Neural signals conveying information about environmental light are transmitted from the retina through the retinohypothalamic tract to the hypothalamic suprachiasmatic nuclei (SCN) [7]. In turn, the SCN transmit information about lighting and circadian time to the pineal gland where the hormone melatonin is synthesized [8]. As a result, daily patterns of light exposure entrain the circadian secretion of melatonin. In most vertebrate species studied, this pattern results in higher levels of melatonin during the dark, night-time hours. In addition, exposure to light of sufficient intensity, wavelength, and duration can acutely suppress high nocturnal melatonin secretion from the pineal gland [9–11]. Once released into the blood vascular system, melatonin is known to be a pluripotent hormone that modulates the circadian system at the level of the SCN as well as numerous peripheral tissues [8, 12]. As melatonin is lipophilic, it readily transfers into all other body fluids [8]. Recent evidence from sheep has supported the theory that an important route of the delivery of the melatonin signal to brain tissue is directly from the pineal gland into the cerebrospinal fluid of the third ventricle [13, 14].

In the past 15 years, there have been fundamental advances in the understanding of photoreceptive input to the circadian and neuroendocrine systems of humans and other mammals. In 2001, two human analytic action spectra identified 446–477 nm as the most potent wavelength region for melatonin suppression [10, 11]. Those data indicated that a novel ocular photosensory system, distinct from the visual rods and cones, is primarily responsible for regulating melatonin in humans. Based on selected monochromatic wavelength comparisons, further human studies indicated that circadian phase-shifting, and autonomic stimulation, as well as the acute effects of light on alertness and vigilance are shifted toward the shorter wavelength, or blue-appearing, part of the spectrum [15–22].

Seminal discoveries have elucidated the basic anatomy and physiology of the photosensory system that supplies input to the circadian and neuroendocrine systems. It is

clear that a small population of widely dispersed retinal ganglion cells is directly responsive to light and projects to the SCN as well as regulatory nuclei in the central nervous system [23, 24]. These intrinsically photosensitive retinal ganglion cells (ipRGCs) have an expansive arbor of dendrites that form a 'photoreceptive net' for circadian phototransduction [25]. The ipRGCs contain melanopsin, a vitamin A photopigment that mediates phototransduction in these cells [26]. Although light detection for circadian, neuroendocrine, and neurobehavioral responses is mediated principally by the ipRGCs, studies on genetically manipulated rodents [27–30], normal monkeys [31], and humans [15, 20, 32], clearly demonstrate that the visual rod and cone photoreceptors also have a role in modulating this physiology.

The goal of the research presented here is to determine the best combination of light wavelengths for use as a lighting countermeasure for circadian and sleep disruption during space exploration, as well as for individuals living on Earth. The specific hypothesis tested in the following studies is that broad spectrum fluorescent light enriched in the short-wavelength portion of the visible spectrum will be more potent for the regulation of pineal melatonin secretion in healthy men and women. The data demonstrate a fluence–response relationship between melatonin suppression and corneal light irradiances for each of three types of fluorescent light. Comparison of these fluence–response curves supports the hypothesis that polychromatic fluorescent light is more potent for melatonin regulation when enriched in the short-wavelength spectrum.

Materials and methods

Hazard analysis

As exposure to ultraviolet radiation or intense visible light within the range of 400–550 nm can induce damage the eye, an assessment of the three lighting systems used in this research was necessary [33, 34]. An independent hazard analysis based on guidelines set by the American Conference of Governmental Industrial Hygienists (ACGIH) and the International Commission on Non-Ionizing Radiation Protection (ICNIRP) was performed to ensure that subjects were not at risk for photochemical retinal injury. All measurements of the experimental light panels were taken at 30 cm, the typical viewing distance of the panels in the study. The primary analysis was completed using three instruments: (i) a Fieldspec hand-held spectroradiometer manufactured by Analytical Spectral Device (Boulder, CO, USA), Model #FSHH 325-1075, for all spectroradiometer measurements; (ii) a radiometer/photometer (Model 1400BL; International Light Technologies, Inc., Newburyport, MA, USA) with a silicon-diode detector head (Model #SEL033), a wide-angle output optic (W#11437) and a filter (Y#27475) to provide photopic illuminance response; and (iii) a Minolta luminance meter (nt°-1), Model LS-100 (Tokyo, Japan), to measure the panel luminance as a cross-check of the spectroradiometric radiance measurements at 30–70 cm. Measurements of each panel also were made with an ultraviolet light detector using an International Light Model 1400A radiometer with model SEL240 (#3682) and Model SEL033 (#3805) detector (with input optic W#6874 and filter UVA #28246) to assure that no hazardous UV radiation was emitted from the surface of the light panels.

Study design

In each of three studies, eight subjects completed a within-subjects fluence–response experiment that tested nine light irradiances of polychromatic lamplight and one dark exposure control night for nocturnal melatonin suppression. The three test lamplights were standard white light 4000 K fluorescent, prototype blue-enriched white 17,000 K fluorescent, and prototype strongly enriched blue (SEB) fluorescent.

Subjects

The healthy females (n = 12) and males (n = 12) in these studies had a mean ± S.E.M. age of 24.3 ± 0.4, had a mean wake up time of 07:49 hr ± 8 min, and signed an approved IRB consent document before participating. All subjects demonstrated normal color vision by both the Ishihara and Farnsworth Munsell D-100 color vision tests (mean ± S.E.M. score of 83.8 ± 67.2). All subjects were in good ocular and physical health as assessed by a neuro-ophthalmologist, good mental health as assessed by a clinical psychologist, and free of signs of substance abuse as determined by urinary toxicological screens. Ten days prior to entry into the protocol, subjects were confirmed for sleep–wake stability with routine bedtime starting at midnight (±30 min) as ascertained by actigraphy (Octagonal Sleepwatch; AMI, Ardsley, NY, USA), filling out daily sleep–wake logs and confirming wake and bed times by calling into a voice mailbox.

Light exposure protocol

Subject pre-experimental adaptation began in the laboratory at 18:00 hr. During this period, subjects stayed upright in chairs engaging in reading, studying, or conversation and were not permitted to sleep. Light levels did not exceed 10 lux at eye level in any direction of gaze. As described elsewhere, each experiment began at midnight when subjects were blindfolded and remained awake and sitting upright in darkness for 120 min [10]. While blindfolded, a blood sample was taken just prior to 02:00 hr and subjects were then exposed to a 90-min light stimulus from 02:00 to 03:30 hr. Before experimental light exposures, blindfolds were removed. Subjects' pupils were not dilated in this study. During light exposure, each subject sat quietly with their eyes open and facing a 119 × 120 cm flat panel of fluorescent lights with their eyes 30 cm from the central portion of the light-emitting surface. The light was patternless and provided full retinal field exposure. At 03:30 hr, a second blood sample was taken. Each subject was exposed to complete darkness from 02:00 to 03:30 hr on their control night and was tested with at least 6 days between each night-time exposure.

Light production and measurement

Polychromatic light stimuli were produced by equipment donated by Philips Lighting (Eindhoven, Netherlands). Test lamps were white 4000 K fluorescent lamps (TL5 HO 54W/830), prototype blue-enriched white 17,000 K fluorescent lamps, and prototype SEB fluorescent lamps housed in Strato Luminaires (Model TPH 710) containing high-speed electronic ballasts. Each luminaire was electronically dimmable down to 40 μW/cm^2. Neutral density filter panels were used to further adjust the intensity of the light levels below 40 μW/cm^2: 0.3 ND (0.3 optical density; 50% transmittance), 0.6 ND (0.6 OD; 25% transmittance), and 0.9 ND (0.9 OD; 12.5% transmittance; Rosco Laboratories, Stamford, CT, USA). Spectroradiometric and photon flux assessments of the wavelengths at the level of subjects' corneas were carried out with a portable spectroradiometer with a fiber optic sensor (FieldSpec Model #FShh 325-1075P; ASD, Boulder, CO, USA). The spectral power distributions of the lamplights are shown in Fig. 1. Routine measurement of the light irradiance (μW/cm^2) was carried out with International Light Radiometer/Photometer 1400A (Newport, MA, USA) with an SEL033 #6857 detector head with an F #23102 filter and cosine correction. All spectroradiometric and radiometric equipment was calibrated annually with a standard lamp traceable to NIST. Light irradiances and illuminances were measured at volunteers' eye level immediately before and after the 90-min exposure. Additional spot measures were taken with a 1° luminance meter (#301749; Minolta Camera Co. Ltd., Tokyo, Japan) each half hour of the exposure to ensure stimulus stability. For each fluence–response study, intensities ranged over a four log unit photon flux range of 10^{10} to 10^{14} photons/cm^2.

Melatonin assay

As detailed elsewhere, radioimmunoassay (RIA) was used to assay plasma for melatonin [10, 35]. Duplicate 200 μL aliquots of each unknown and control sample were extracted into chloroform. Then, chloroform was removed by centrifugation, samples were resuspended in assay buffer, washed twice with petroleum ether and evaporated to dryness. Samples then were resuspended with deionized water, incubated for 48 hr with radiolabelled ligand and R1055 antiserum, precipitated by centrifugation, and radioactivity was quantified [10, 35]. This RIA had a minimum detection limit of 0.5–2.0 pg/mL.

Calculation of effective rod, cone, and melanopsin photoreceptor illuminances

To calculate human rod, cone, and melanopsin photoreceptor illuminances, the lamp SPDs were entered into a toolbox worksheet that is a software model for human photoreception freely available online [36]. The SPDs for the experiments shown here were imported into the worksheet in 1 nm increments between 378 and 782 nm. Per toolbox instructions, all raw negative SPD values were changed to zero.

Statistics

Two-tailed, Students' *t*-tests were used to assess significance of raw melatonin change from 02:00 to 03:30 hr. Raw melatonin data were then converted to % melatonin change scores and % control-adjusted change scores as described elsewhere [10]. Sets of pre-exposure melatonin values and % control-adjusted melatonin change scores were analyzed with one-way, repeated-measures ANOVA. Significant differences between groups were assessed with post hoc Fisher PLSD test with alpha of 0.05. The fluence–response curves for light exposures were calculated and fit using Origin 8.0 (OriginLab Corp., Northampton, MA, USA). The curves were fit to an unconstrained parametric model with the melatonin response (Y) to the corneal irradiance (X) is predicted by: a theoretical initial Y-response for the curve (A_1); the theoretical final Y-response ('infinite' dose) for the curve (A_2); the dose producing a response halfway between A_1 and A_2 (X_{50}); and the slope estimator for the curve between A_1 and A_2 (p). These compose the equation:

$$Y = \frac{A_1 - A_2}{1 + (X/X_{50})^p} + A_2$$

The curves were tested for fit of the data by coefficient of correlation (R^2).

Results

Based on national and international safety criteria, the hazard analysis determined that all intended study exposures used in this study were safe [33, 34]. The radiance measurements did not exceed the long-term limit of 10 mW/(cm^2 sr). Spectral weighting of the 4000 K, 17,000 K and SEB spectral power distributions with the blue-light hazard or B(λ) function provided a maximum effective blue-light radiance value of 0.0094, 0.061, and 0.41 mW/(cm^2 sr), respectively. Thus, the SEB panel, the most blue-enriched of the three fluorescent panels, was <5% of the ACGIH and ICNIRP limits [33, 34].

Selected light stimuli characteristics (corneal irradiances, photon fluxes and illuminances) are provided in Table 1 along with measured mean pupil sizes and calculated mean retinal irradiances.

ANOVA demonstrated a significant effect of light on pupil diameter for the 4000 K exposures (F = 118.3, d.f. = 8, P < 0.0001), the 17,000 K exposures (F = 102.4, d.f. = 8, P < 0.0001), and the SEB exposures (F = 104.0, d.f. = 8, P < 0.0001). The Fisher PLSD test showed that pupil diameters were significantly larger when exposed to the lowest (1 through 18 μW/cm^2) versus higher (40 through 734 μW/cm^2) 4000 K irradiances. For the 17,000 K exposures, pupil diameters were significantly larger with the lowest (1, 5, and 10 μW/cm^2) versus higher irradiances (20 through 800 μW/cm^2). Finally, for the SEB exposures, pupil diameters were significantly larger with the lowest (0.8 and 4 μW/cm^2) versus higher irradiances (6 through 1500 μW/cm^2).

The top graph in Fig. 2 compares mean (+ S.E.M.) pre- and postplasma melatonin values for each study

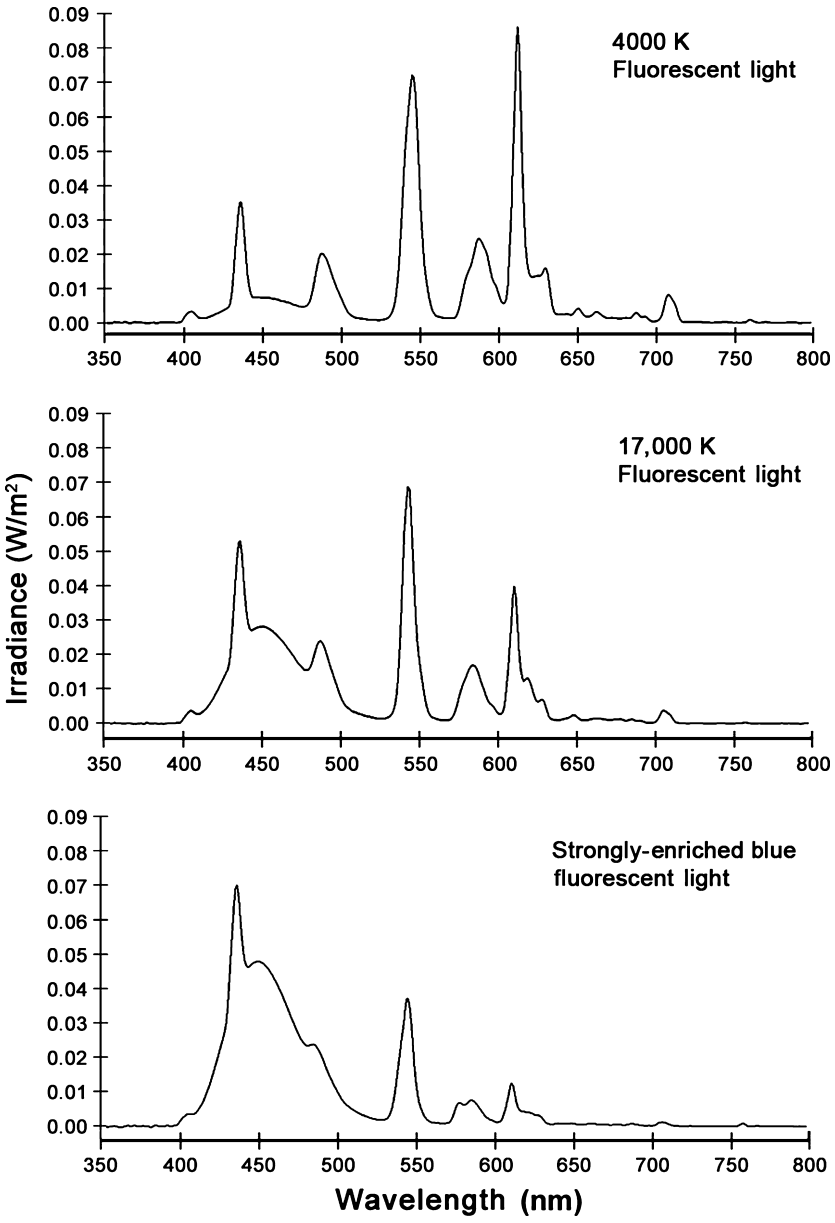

Fig. 1. These graphs show the SPD of the polychromatic light sources. The top SPD is from a standard white fluorescent lamp (4000 K CCT). The middle SPD graph is from a prototype white-appearing, blue-enriched lamp (17,000 K CCT). The bottom SPD is from a prototype SEB lamp. CCT, correlated color temperature; SEB, strongly enriched blue; SPD, spectral power distribution.

night from the 4000 K light exposures. All irradiances at or above 1 $\mu W/cm^2$ significantly suppressed melatonin ($P < 0.05$ to $P < 0.005$). Percent control-adjusted melatonin change scores presented in the bottom graph of Fig. 2 showed a significant effect of light irradiance on melatonin suppression ($F = 13.24$, d.f. = 8, $P < 0.0001$). Increasing irradiances of 4000 K light exposure evoked progressively larger melatonin suppressions. The Fisher PLSD test showed that higher irradiances of 4000 K light (734 and 274 $\mu W/cm^2$) elicited significantly stronger melatonin suppression than irradiances at or below 80 $\mu W/cm^2$. The 80 $\mu W/cm^2$ irradiance of 4000 K light elicited a significantly stronger melatonin suppression than lower irradiances (8.34, 3.81, and 1 $\mu W/cm^2$), while 39.5 and 18 $\mu W/cm^2$ elicited significantly stronger melatonin suppression than the lowest irradiance of 0.8 $\mu W/cm^2$.

For the 17,000 K light exposures, comparison of the mean pre- and postplasma melatonin values shows that all corneal irradiances at or above 15 $\mu W/cm^2$ significantly suppressed melatonin ($P < 0.05$ to $P < 0.005$). For the 17,000 K light exposures, percent control-adjusted melatonin change scores showed that there was a significant effect of light irradiance on melatonin suppression ($F = 12.48$, d.f. = 8, $P < 0.0001$). Increasing irradiances of 17,000 K light evoked progressively larger melatonin suppressions. Fisher PLSD tests showed that the 800 $\mu W/cm^2$ corneal irradiance elicited significantly stronger melatonin suppression than irradiances at or below 50 $\mu W/cm^2$, while the 300 $\mu W/cm^2$ irradiance produced a significantly stronger melatonin suppression than irradiances at or below 15 $\mu W/cm^2$. The intermediate 17,000 K light irradiances of 50 and 25 $\mu W/cm^2$ evoked significantly stronger melatonin suppressions than irradiances at or below

Table 1. Selected target corneal irradiances, measured corneal illuminances, corneal photons (380–760 nm), measured pupil sizes, and calculated retinal irradiances of light stimuli

Fluorescent stimulus	Corneal irradiance (μW/cm^2)	Corneal illuminance (lux)	Photon flux (photons/cm^2/s)	Mean pupil diameter (mm)	Calculated retinal irradiance (μW/cm^2)
4000 K	734	2874	2.03×10^{15}	2.72	4.24
4000 K	40	135	1.14×10^{14}	4.19	0.54
4000 K	11	23	2.64×10^{13}	4.72	0.19
17,000 K	800	2511	1.84×10^{15}	3.02	5.90
17,000 K	25	71	4.45×10^{13}	4.33	0.37
17,000 K	10	27	1.78×10^{13}	4.83	0.18
Strong Blue	1500	2397	3.28×10^{15}	2.28	6.38
Strong Blue	58	91	6.71×10^{14}	3.39	0.52
Strong Blue	10	13	1.16×10^{13}	4.74	0.18

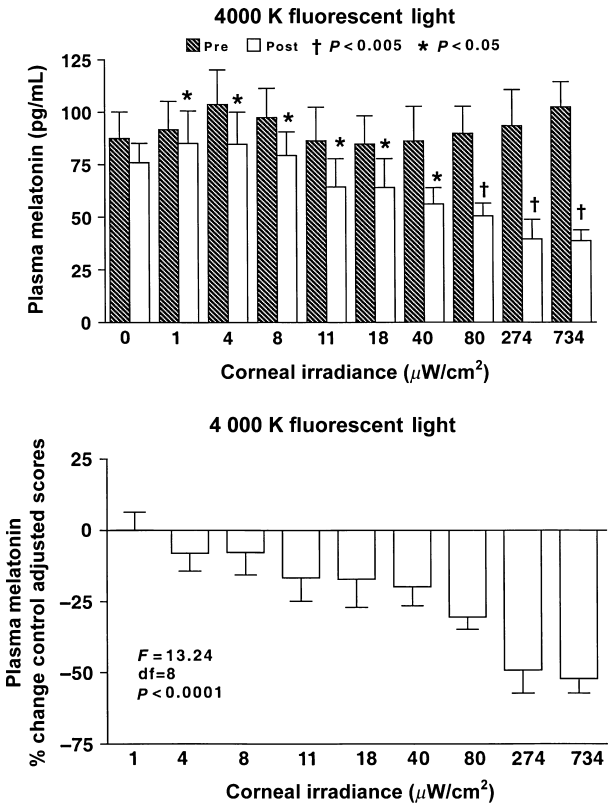

Fig. 2. The top graph compares the mean (+ S.E.M.) pre- and postplasma melatonin values for the 4000 K light exposures. Percent control-adjusted melatonin change scores from these data are presented in the bottom graph.

25 μW/cm^2, and irradiances at or below 10 μW/cm^2, respectively. Lastly, 20 μW/cm^2 elicited a stronger melatonin suppression than 10 and 1 μW/cm^2; while 15 and 5 μW/cm^2 produced significantly stronger suppressions than 1 μW/cm^2.

Comparison of the mean pre- and postplasma melatonin values of the SEB light exposures shows that all irradiances at or above 17.4 μW/cm^2 significantly suppressed melatonin ($P < 0.05$ to $P < 0.005$). For the SEB percent control-adjusted melatonin change scores, there was a significant effect of light irradiance on melatonin suppression ($F = 9.43$, d.f. = 9, $P < 0.0001$). Increasing irradiances of SEB light exposure evoked progressively larger melatonin suppressions. Fisher PLSD tests showed that the higher irradiances of SEB light (1500 and 323 μW/cm^2) elicited significantly stronger melatonin suppression than irradiances at or below 22 μW/cm^2. The corneal irradiance 58.4 μW/cm^2 elicited significantly stronger melatonin suppression than lower irradiances at or below 10 μW/cm^2, while 22 μW/cm^2 elicited significantly stronger melatonin suppression than irradiances at or below 6 μW/cm^2. Finally, 17.4 μW/cm^2 elicited significantly stronger melatonin suppression than irradiances of 6 and 4 μW/cm^2.

Fig. 3 shows the mean (±S.E.M.) percent change control-adjusted melatonin data from the three studies presented above plotted on a log scale of corneal irradiance along with the best-fit, sigmoidal curve. No mathematical constraints were placed on this family of curves. The coefficients of correlation for the 4000 K, 17,000 K, and SEB curves were 0.96, 0.85, and 0.95, respectively.

The curves illustrated in Fig. 3 show that there is a progressive steepening of the slopes (P value) of the curves with greater enrichment with blue light. Further, the pseudo-linear portions of both the 17,000 K and SEB curves are shifted to the left in comparison to the 4000 K curve. That shift is quantified by comparison of the curve's X_{50} parameters (4000 K = 95.81, 17,000 K = 16.40, and SEB = 21.00). Hence, approximately six times as much light is needed to evoke a half-saturation response from the 4000 K light versus the 17,000 K light. Similarly, approximately five times as much light is needed to evoke a half-saturation response from the 4000 K light versus the SEB light.

The fluence–response curves in Fig. 3 were not mathematically constrained. In action spectroscopy, it is not unusual to constrain one or more of the elements (A_1, A_2, p) of the parametric model described above. Including the unconstrained curve fits in Fig. 3, seven different parametric models for curve fitting the melatonin data were tested. For example, one constrained model that was tested set $A_1 = 0$. The logic of that constraint is that darkness (a corneal irradiance of 0) would elicit 0% melatonin suppression. The curve fits of all models all had relatively high coefficients of correlation (R^2 ranged from 0.80 to 0.97). The resulting X_{50} parameters resulting from application of the different models also varied in their ranges (4000 K = 65.80–95.81, 17,000 K = 9.57–25.40, and SEB

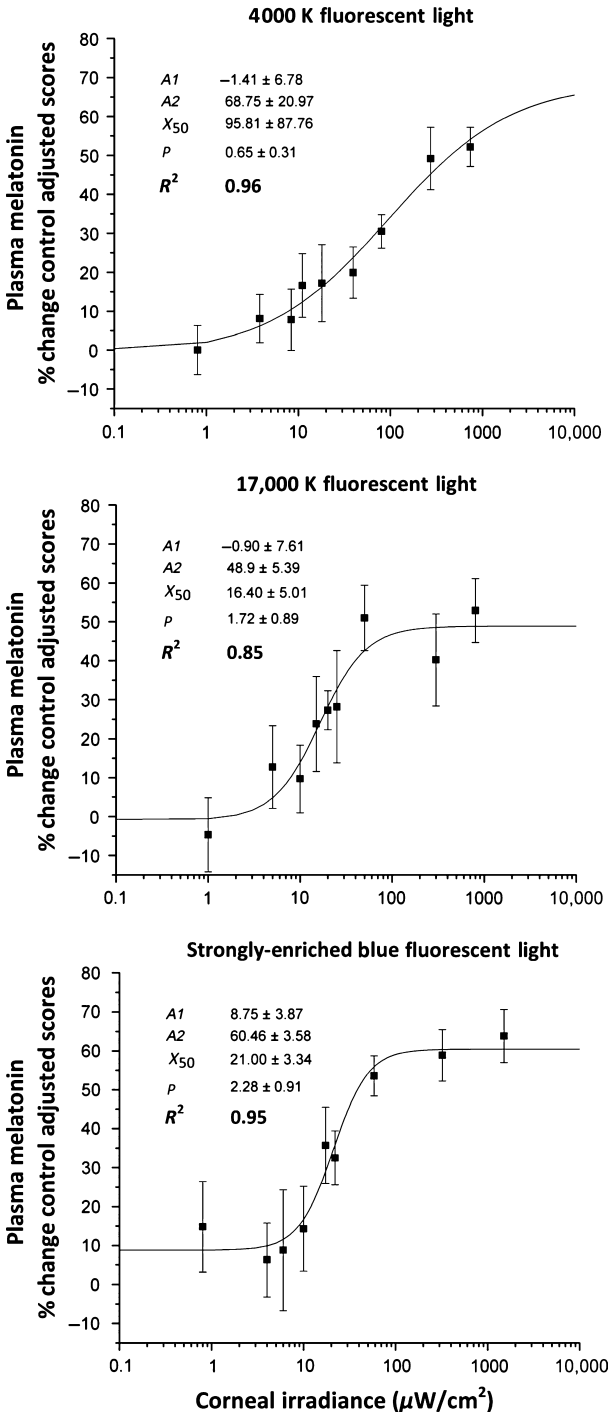

Fig. 3. Each fluence–response curve represents data collected from separate cohorts of eight healthy subjects after exposure to different types of polychromatic fluorescent light. The curve fit parameters and coefficient of correlation (R^2) are included in the upper left portion of each graph.

= 17.80–22.70). In every case, when a single parametric model was applied across all sets of fluence–response data, the blue-enriched 17,000 K and SEB light elicited smaller half-saturation responses compared to the 4000 K light. Across these different parametric models, a range of approximately three to seven times as much irradiance is needed to evoke a half-saturation response from the 4000 K light versus the 17,000 K light. Similarly, approximately three to five times as much light is needed to evoke a half-saturation response from the 4000 K lamp versus the SEB light.

The effective rod, cone, and melanopsin photoreceptor irradiances and the total irradiances and photopic illuminances were calculated as described above [36]. The values in Table 2 were derived from the SPDs shown in Fig. 1 with photon fluxes of 9.18 or 9.19 $\times$ 10^{14} photons/cm^2/s.

Discussion

This study tested three types of broad spectrum fluorescent lamps that differed in their relative emission of light in the short-wavelength end of the visible spectrum between 400 and 500 nm. A hazard analysis, based on national and international eye safety criteria, determined that all light exposures used in this study were safe [33, 34]. The melatonin data comprise three full fluence–response relationships between graded exposures from the different types of fluorescent light and melatonin suppression in healthy humans. Comparison of the three fluence–response curves supports the hypothesis that polychromatic fluorescent light is more potent for melatonin regulation when enriched in the short-wavelength portion of the visible spectrum.

Action spectroscopy is a powerful tool for revealing the underlying physiology of phototransduction. The development of fluence–response curves using monochromatic light (≤15 nm half-peak bandwidths) is fundamental for creating action spectra [37–39]. That approach has been used extensively for clarifying the sensitivity of neuroendocrine and circadian responses to light [20, 23, 31, 39, 40]. Similar to the fluence–response curves developed with monochromatic light for melatonin suppression in humans, the polychromatic fluence–response curves presented here have strong fits to four parameter parabolic curves [10, 11]. There is, however, a major methodological difference between the experiments reported here versus the earlier human melatonin action spectra. Specifically, the volunteers' eyes were not pharmacologically dilated in the current studies. As shown in Table 1, the mean pupillary size varied across different irradiances and lamp types. Pupil size, in part, modifies retinal irradiance. Lighting countermeasures for circadian and sleep disruption during space exploration, as well as for individuals living on Earth, will be used when individuals have freely reactive pupils.

It is useful to compare the results reported here to two other full-range human melatonin suppression fluence–response curves in subjects with freely reactive pupils [41, 42]. In one study, the light stimuli were produced by narrow bandwidth blue solid-state light-emitting diodes (LEDs, 469 nm peak, 26 nm half-peak bandwidth) with an exposure system identical to the one described here [41]. The calculated irradiance for a half-saturation response (X_{50}) from the blue LEDs was 14 μW/cm^2 – similar to the X_{50}s of 16 and 21 μW/cm^2 for the 17,000 K and SEB fluorescent lamps, respectively. In contrast, the 4000 K lamps had a much higher X_{50} of 96 μW/cm^2.

Table 2. Calculated irradiances, photopic illuminances v(λ), and human photopigment illuminances relative to three broad spectrum lights [32]

	Radiometric and Photometric Values (380–780 nm inclusive)			Retinal Photopigment Weighted Illuminances (α-optic lux)				
	Photon Flux (photons/cm²/s)	Irradiance (μW/cm^2)	Photopic Illuminance (lux)	S Cone	Melanopsin ipRGC	Rod	M Cone	L Cone
4000 K Light	9.18E+14	329	1141	688	711	833	1011	1107
17,000 K Light	9.19E+14	357	884	1713	1212	1119	982	889
SEB Light	9.18E+14	379	581	2682	1613	1298	872	647

Together, these results indicate that blue-enriched polychromatic light elicits stronger melatonin suppression compared to equivalent intensities of light with lower emissions in the blue-appearing portion of the visible spectrum. Importantly, the data indicate that there may be limits to blue-enriching a light source for evoking stronger melatonin regulation; the half-saturation responses from the SEB and 17,000 K exposures are roughly similar even though the SEB lamps emitted higher concentrations of energy in the blue portion of the spectrum compared to the 17,000 K lamps.

Another study reported fluence–response curves for both phase-shifting the melatonin rhythm and suppressing nocturnal melatonin relative to cool white fluorescent light exposures [42]. The study conditions and light exposure techniques were very different from the fluence–response results discussed above. Notably, light exposures were significantly longer (390 min versus 90 min). Further, subjects were adapted to an ambient illuminance of ≤10 lux for 50 hr, had an 8-hr sleep opportunity in darkness, and then a 5-hr period in ≤10 lux before the experimental light exposures. The resultant melatonin phase-shift and suppression fluence–response curves yielded X_{50} values of 36 and 32 μW/cm^2, respectively. It is likely the exposure duration and lengthy dim light/darkness adaptation contributed greatly to the differences between the X_{50} value of 96 μW/cm^2 relative to the 4000 K data reported here. It has been shown that increasing light exposure duration from 30 to 120 min increases the melatonin suppression in human subjects [43]. Similarly, prior dim light exposure has been shown to increase the sensitivity to light in melanopsin ganglion cells in rats as well as for melatonin suppression in humans [32, 44–46].

A variety of behavioral and physiological changes relative to correlated color temperature (CCT) of broad spectrum fluorescent lights have been measured [47, for review]. Generally, higher color temperature lamps emit more energy in the blue part of the visible spectrum than lower color temperature lamps [48]. Lamps with higher CCT were found to evoke a stronger melatonin suppression compared to lamps with lower CCT in healthy humans [49–51]. Additionally, compared to low CCT light, higher CCT light was observed to have a more potent effect on reducing the nocturnal fall of core temperature, decreasing body temperature change to cold challenge, and increasing the morning rise in core temperature [50, 52, 53]. Furthermore, EEG frequency has been shown to increase under high CCT as compared to lower CCT illumination [54]. Finally, a study on the effects of illumination prior to sleep showed that deep sleep was reduced under high CCT compared to low CCT during the first half of sleep [55]. This composite literature consistently demonstrates that higher CCT light induces stronger acute neuroendocrine and neurobehavioral effects than lower CCT light in healthy subjects. Those findings are generally consistent with the analytical action spectra that demonstrate the blue-appearing portion of the visible spectrum is more potent for circadian, neuroendocrine, and neurobehavioral regulation.

Only three studies have compared cool white fluorescent light versus blue-enriched fluorescent light (17,000 K) for phase-shifting the circadian system in humans. Two studies compared equal photon fluxes of 4100 K versus 17,000 K light for phase-delaying or phase-advancing dim light melatonin onset (DLMO) [56, 57]. Both studies showed that each type of light elicited strong DLMO phase-shifts with no significant difference between groups. Those studies used relatively high, potentially saturating, light levels that are commonly used for evoking phase-shifts (4.2×10^{15} photons/cm^2/s, or 4000–5000 lux). A third study compared equal photon doses of lower light levels (1×10^{14} photons/cm^2/s or 134–129 lux) of 4000 K versus 17,000 K light for eliciting a DLMO phase-delay in healthy subjects [58]. Although the 17,000 K light caused a greater mean phase-delay shift of 2.1 hr compared to 4000 K light that elicited a 1.7 delay, the difference was not statistically significant. These studies indicate that blue-enriched fluorescent light has greater potency for eliciting acute biological and behavioral responses but longer term effects like circadian phase-shifting does not reflect that same sensitivity. Further research is needed to clarify such differences between short- and long-term effects of polychromatic light.

Measured radiometric and photometric values for the studies' lighting stimuli are provided in Fig. 1 and Table 1. Currently, there is not a standardized, single measurement unit available for quantifying light that regulates the circadian, neuroendocrine and neurobehavioral effects of light. A recent consensus position was developed across many of the laboratories that have studied wavelength regulation of the biological and behavioral effects of light in humans and other species for best practices for measuring and reporting light stimuli [36]. With that consensus, a freely available web-based toolbox was provided that permits calculation of the effective irradiance for each of the human ipRGC, cone and rod photoreceptors that are capable of driving physiological effects [36]. Table 2 provides the computed effective photoreceptor illuminanc-

es of the three polychromatic lights used in this study. Such data are intended for making comparisons of polychromatic lights of different spectral quality significantly more traceable [36].

Examining the calculated photoreceptor illuminances, it is clear that lamplight that is progressively enriched with short wavelengths is predicted to have increasingly higher S cone, melanopsin ipRGC, and rod weighted illuminances. Conversely, those same lamplights have progressively lower M cone and L cone weighted illuminances. Given that the ED_{50} for melatonin suppression of both 17,000 K and SEB lamplight were five times smaller than that of the 4000 K lamplight, the empirical melatonin suppression data support the hypothesis that polychromatic light with increased emissions of short wavelengths has increased potency for acute regulation of the pineal gland. The melatonin data, however, suggest that there are limits to increasing potency of light with short wavelengths as the SEB light did not have an ED_{50} lower than the 17,000 K light.

Although it would be desirable to use a single unit for quantifying light that elicits circadian, neuroendocrine, and neurobehavioral responses, this is not currently feasible. Abundant evidence shows that the melanopsin ipRGCs are anatomically and functionally interconnected with the rods and cones that support vision [20, 27–32, 36, 40, 59, 60]. Light-induced physiological responses reflect input from all of the retinal photoreceptor classes, with the relative importance of each being highly labile within and between response types. Consequently, the spectral sensitivity of this photoreceptive system is fundamentally context dependent [15, 17, 20, 29–32, 36, 40, 59, 60].

The lack of a consistent and adequate method of quantifying light makes it challenging to replicate experimental conditions or to compare across studies. For this nascent field of research to mature, different groups of investigators need to use commonly accepted metrics for reporting spectral response functions to be able to pool results such as those shown in Table 2. As published data using this measurement system accumulate, it will then be possible to generate testable hypotheses that predict the spectral characteristics for a targeted physiological or behavioral response to light.

There are limitations to the data presented here. First, each study employed young adults. Results from studies with older adults would differ significantly due to age-related differences such as ocular media transmission [61, 62]. Second, although each fluence–response curve was developed with a complete within-subjects study design, three separate cohorts of subjects were studied. An ideal comparison of the potency across three lamplights would have employed the same subjects for all three curves. That is logistically challenging, however, due to subject compliance and drop out. No published human neuroendocrine studies yet have provided three full-range fluence–response curves within a single subject cohort. Finally, the light exposure technique involved constant 90-min full retinal field exposures. Such exposures are optimum for comparing the potency of the three types of lamplight. When used in daily applications such as the home, workplace, or spaceflight environments, exposure conditions rarely would be full retinal field or continuous. Studies in a variety of architecturally illuminated spaces are needed to define the optimum illuminances and irradiances that support vision as well as the biological and behavioral efficacy of built-in lighting.

Risks for the health and safety of astronauts and ground control workers include disturbed circadian rhythms and sleep loss. Such problems may cause impaired alertness, loss of concentration, and diminished performance [5, 6, 63, 64]. The broader research goal was to determine the best combination of wavelengths for a lighting countermeasure for circadian and sleep disruption during space exploration, and individuals on Earth. The data presented here provide a first step toward that aim. NASA has used high illuminances of white fluorescent light as a prelaunch countermeasure for circadian disruption in astronauts [5]. Solid-state lighting now provides the potential to provide astronauts light therapy during spaceflight. The retrofit of the International Space Station (ISS) fluorescent lighting system to solid-state lighting is scheduled to begin in 2016 [65]. A recent study on the Mars Phoenix Lander ground crew demonstrated that a blend of countermeasures including exposure to blue solid-state light effectively improved alertness and circadian entrainment [6]. Initial tests of have probed the ISS lighting system's capacity for supporting astronaut vision and regulating melatonin [66]. Lighting countermeasures are promising for general domestic use in architectural applications or clinically, such as treating specific circadian disorders. Results from two field studies testing 17,000 K fluorescent lamps in the work places indicate that this lamplight improved employee alertness and well-being [67, 68]. Now the door is open to future lighting designs of building interiors that address the traditional lighting values along with fostering benefits to human health and well-being.

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Author contributions

George Brainard and John Hanifin were responsible for study design, data acquisition, analysis, and interpretation,

as well as the original manuscript draft. Benjamin Warfield, Marielle Stone, Mary James, Melissa Ayers, Alan Kubey, Brenda Byrne, and Mark Rollag were each involved in data acquisition, analysis, and interpretation, as well as critical manuscript revision. All authors have approved the submitted version of the manuscript.

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