Extending the Circadian Phototransduction Model: Quantifying the Effect of Light Exposure Duration and Age on Nocturnal Melatonin Suppression in Humans

Project Report

Prepared for the Jim H. McClung Lighting Research Foundation

Prepared by the Lighting Research Center,
Rensselaer Polytechnic Institute

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July 25, 2018

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1. Background

Lighting for most indoor spaces has been designed to meet visual requirements and address energy-conservation concerns. [1] However, past studies have shown that appropriately timed light exposure can serve as a powerful non-pharmacological tool for mitigating irregular sleep-wake patterns and promoting circadian entrainment, [2-4] and that exposure duration can influence the efficacy of light therapy. [5-8] Research to date has primarily and successfully focused on the spectral sensitivity of the human circadian system, [9-13] but a lack of resolution in respect to the temporal characteristics of photic stimuli for promoting circadian entrainment and health continues to pose a key challenge to the development of more comprehensive light-treatment standards.

Published psychophysical studies clearly demonstrate that light exposures can shift circadian phase, and that exposure to light at night can suppress secretion of the hormone melatonin, a well-established marker of the circadian system. [4,6,14-16] Using empirical, light-induced nocturnal melatonin suppression data from Brainard *et al.* [17] and Thapan et al., [18] Rea *et al.* [12,19] have proposed a model of human circadian phototransduction based on fundamental knowledge of retinal neurophysiology and neuroanatomy. Operationally, the model provides a framework for depicting how the classical photoreceptors (i.e., rods and cones) provide input to the intrinsically photosensitive retinal ganglion cells (ipRGCs), which are the main conduit of electrical signals from the retinae to the master clock in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus.

Mathematically, for any light source, the model converts the spectral irradiance at the cornea into circadian light (CL_A) (Equation 1), reflecting the spectral sensitivity of the circadian system, and then transforms it into a CS value reflecting the absolute sensitivity of the circadian system (Equation 2). Thus, CS is a measure of the *effectiveness* of the retinal light stimulus for the human circadian system from threshold (CS = 0.1) to saturation (CS = 0.7). The model does not take the light exposure's duration into account, however, and assumes a duration of 1 h, which is a limitation of the model that was addressed by the present study.

$$CL_A = \begin{cases} 1548\left[\int Mc_\lambda E_\lambda d\lambda + \left(a_{b-y}\left(\int \frac{S_\lambda}{mp_\lambda} E_\lambda d\lambda - k\int \frac{V_\lambda}{mp_\lambda} E_\lambda d\lambda\right) - a_{rod}\left(1 - e^{\frac{-\int V'_\lambda E_\lambda d\lambda}{RodSat}}\right)\right)\right] & \text{if } \int \frac{S_\lambda}{mp_\lambda} E_\lambda d\lambda - k\int \frac{V_\lambda}{mp_\lambda} E_\lambda d\lambda \geq 0 \\ 1548\int Mc_\lambda E_\lambda d\lambda & \text{if } \int \frac{S_\lambda}{mp_\lambda} E_\lambda d\lambda - k\int \frac{V_\lambda}{mp_\lambda} E_\lambda d\lambda < 0 \end{cases} \quad (1)$$

CL_A: circadian light. The constant, 1548, sets the normalization of CL_A so that 2856 K blackbody radiation at 1000 lux has a CL_A value of 1000.
E_λ: light source spectral irradiance distribution
Mc_λ: melanopsin (corrected for crystalline lens transmittance)
S_λ: S-cone fundamental
mp_λ: macular pigment transmittance
V_λ: photopic luminous efficiency function
V'_λ: scotopic luminous efficiency function
RodSat: half-saturation constant for bleaching rods = 6.5 W/m²
k = 0.2616
a_{b-y} = 0.7000
a_{rod} = 3.3000

$$CS = 0.7 - \frac{0.7}{1 + \left(\frac{CL_A}{355.7}\right)^{1.1026}} \quad (2)$$

The present study set out to investigate recent findings indicating that the same light stimulus can differentially affect the circadian systems of those from different age groups, such as adolescents and adults. [8,20-22] Age-related anatomical changes affecting the transmission of light through the crystalline lens have been extensively documented. [23-25] As the eye ages, the crystalline lens absorbs more light—especially in the short-wavelength region, where the circadian system is maximally sensitive—and pupil area decreases, resulting in a reduction of retinal light exposure.[26,27] Inherently progressive neural changes such as a decline in the number of photoreceptors also have the potential to decrease circadian responsiveness to photic stimuli. [28] Our previous work investigating the impact of conventional indoor white light sources [8] and self-luminous devices [20,22] on melatonin suppression in different age groups suggests an enhanced sensitivity to light at night, particularly short-wavelength light, among adolescents. This hypothesis is well supported by Crowley *et al.*, [29,30] whose studies showed that the endogenous circadian pacemaker's temporal alignment in adolescents can be quite different from that in older individuals due to the maturation of biological processes regulating sleep–wake systems and alteration by psychosocial demands.

Thus, the goals of the present study were to better understand the effect of extended light exposure durations on the suppression of melatonin and to document age-related changes in circadian sensitivity within adolescents and middle-aged adults through the systematic collection of melatonin data. The results of this study will be used to extend the Rea *et al.* model of circadian phototransduction [12,19] by incorporating additional factors of exposure duration and age to better predict effective circadian stimulus. These results will also help to refine our understanding of the threshold for light's effects on the body's production of melatonin.

2. Materials and Methods

2.1. Participant selection

The study's 32 participants (17 females and 15 males) ascribed to 2 age groups, adolescents and adults. The 16 adolescent participants (8 females and 8 males) ranged in age from 13 to 18 years, with a mean ± standard deviation (SD) age of 15.9 ± 1.1 years. The 16 adult participants (9 females and 7 males) ranged in age from 24 to 55 years, with a mean ± SD age of 42.4 ± 10.9 years. The mean ± SD Munich Chronotype Questionnaire scores [31] recorded for the adolescents and adults were 3.4 ± 1.4 and 2.2 ± 1.5, respectively, which suggests that both participant groups were neither extreme larks (early persons) nor extreme owls (late persons). All participants were prescreened for major health problems such as bipolar disorder, seasonal depression, cardiovascular disease, diabetes, and high blood pressure. Participants were excluded from the study if they were taking over-the-counter melatonin or any prescription medications (e.g., blood pressure medicine, antidepressants, sleep medicine, or beta-blockers). They were also excluded if they reported any type of eye disease (e.g., cataracts, glaucoma, etc.).

Given that all participants were either attending school or regularly employed, they were able to follow a consistent sleep–wake schedule (bedtimes no later than 23:00 h and wake times no later than 07:30 h) during the week preceding each study night to maintain their melatonin circadian rhythm. Compliance for the adolescent participants was nonetheless verified using digital wrist-

worn actigraphs (Actiwatch 2, Philips Respironics, Murrysville, PA, USA). Participants were also required to refrain from caffeine consumption for 12 h prior to the start of each study night. None of the participants reported difficulties in complying with the schedule or sleep-related disturbances over the course of the study.

This study conformed to 45 CFR 46 and international ethical standards [32] and was reviewed, approved, and monitored by Rensselaer Polytechnic Institute's Institutional Review Board. Informed consent was obtained from all study participants and/or their legal guardians.

2.2. Experimental conditions

Over the course of the study, all participants were exposed to 2 spectrally distinct white light sources with rated correlated color temperatures (CCTs) of 2700 K and 6500 K (Figure 1). Each spectrum was delivered at 4 photopic light levels (40–1000 photopic lux) that were designed to provide equivalent circadian stimulus (CS) between the 2 spectra at each light level. Figure 2 shows the 4 target CS levels predicted for a 1-h exposure to each of the 2 light sources.

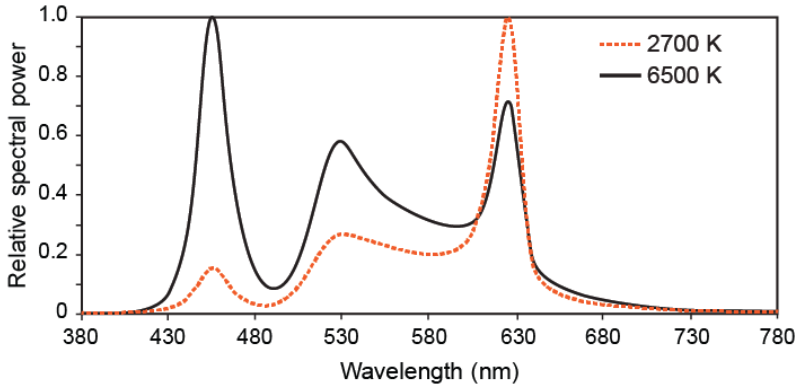

Figure 1. The spectral power distributions of the rated 2700 K and 6500 K LED white light sources used in the study.

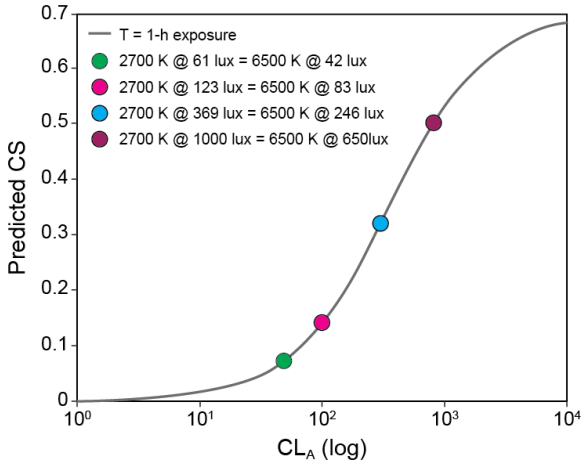

Figure 2. The target CL_A and CS values for the study's lighting conditions for a 1-h exposure, predicted using the Rea et al. model of circadian phototransduction. [12]

The 10-week study was conducted in two 5-week phases. Phase 1 delivered the 2700 K and 6500 K sources at 2 high light levels each, providing target CS levels of 0.50 and 0.30 at participants' eyes, and Phase 2 delivered the 2700 K and 6500 K sources at 2 lower light levels each, providing target CS levels of 0.14 and 0.07 at participants' eyes (see Protocol). For both phases, the participants reported to the laboratory on 5 nights, each separated by at least 1 week to allow for a wash-out period between the conditions. In addition to the 4 intervention nights, all participants were exposed to a dim-light control night that provided a baseline observation of the participants' natural rise in melatonin levels over the course of the night.

2.3. Lighting apparatus

The stimulus for both white light sources was provided and controlled using RGB color-tunable, linear LED light bars (G2, High Output Linear Accent, Ketra, Austin, TX, USA) that were pre-programmed for the desired output modes and mounted on participants' desks (Figure 3). Spectrally neutral diffusers covered the luminaires to eliminate potential glare and provide a uniform light distribution. The light stimulus was calibrated using a tripod-mounted illuminance meter (Model X-91 Broadband Lightmeter, Gigahertz-Optik, Haverhill Rd, Amesbury, MA, USA) to verify the light levels at participants' eyes. Light levels for the target CS levels were computed using the LRC's open-access online CS calculator.

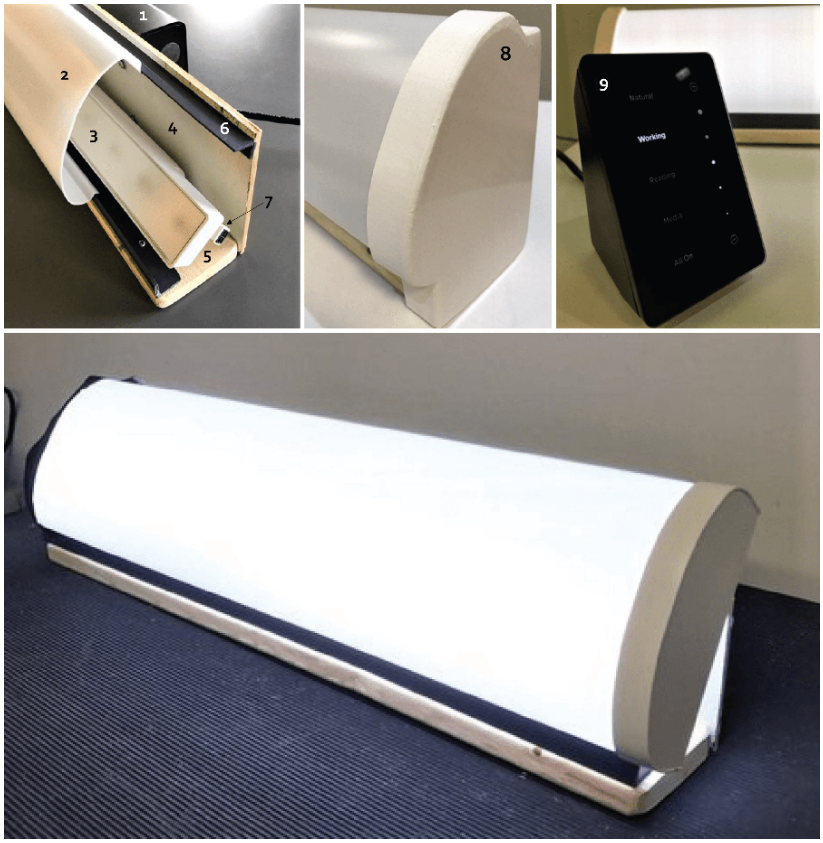

Figure 3. Components of the desktop luminaire that was custom-built by the LRC for use in this study (top): (1) satellite link controller, (2) light diffuser, (3) LED linear accent, (4) plywood housing back, (5) pine board base, (6) ½-in. × 1-in., PVC 90° angle (×2), (7) connector cable, (8) installed endcap, (9) touchpad interface. A prototype of the assembled device is shown in operation below.

2.4. Data recording equipment

To provide better estimates of the retinal light exposures experienced under the experimental conditions, each participant was provided with lensless eyeglasses frames fitted with a Daysimeter that recorded the light stimulus at 30-s intervals throughout the 3-h light exposure. The CL_A [19,33] levels were calculated from the Daysimeter data following Equation 1, using Matlab software (MathWorks, Natick, MA, USA). Participants' mean CS exposures were calculated following Equation 2, based on the Rea *et al.* model. [12,19] The photometric characteristics of the experimental lighting interventions are provided in Table 1. During each 3-h data collection period, light levels at the eye were also spot-checked hourly using a spectrometer (Model USB650 Red Tide Spectrometer, Ocean Optics, Winter Park, FL) and monitored continually using an illuminance meter. The photometric characteristics of the experimental lighting interventions as measured by the spectrometer are provided in Table 2.

Table 1. Photometric characteristics of the lighting interventions as measured and derived from Daysimeter data and the Rea et al. model of circadian phototransduction. [12]

Target CS	Light source (rated CCT)	Adolescents		Adults	
		Photopic Illuminance (Mean ± SD lux)	Predicted CS[a] (Mean ± SD)	Photopic Illuminance (Mean ± SD lux)	Predicted CS[a] (Mean ± SD)
0.3	2700 K	368 ± 42	0.31 ± 0.05	431 ± 73	0.35 ± 0.08
	6500 K	257 ± 34	0.33 ± 0.06	275 ± 34	0.34 ± 0.06
0.5	2700 K	1140 ± 113	0.52 ± 0.13	1181 ± 95	0.52 ± 0.11
	6500 K	665 ± 64	0.50 ± 0.11	694 ± 65	0.51 ± 0.11
0.07	2700 K	74 ± 12	0.09 ± 0.01	76 ± 9	0.09 ± 0.01
	6500 K	50 ± 7	0.08 ± 0.01	50 ± 4	0.09 ± 0.01
0.14	2700 K	140 ± 24	0.16 ± 0.03	147 ± 14	0.16 ± 0.01
	6500 K	95 ± 14	0.16 ± 0.02	96 ± 7	0.16 ± 0.01

Note: (a) The predicted CS values are based on a 1-h exposure.

Table 2. Photometric characteristics of the lighting interventions as measured and derived from spectrometer data and the Rea et al. model of circadian phototransduction. [12]

Target CS	Light source (rated CCT)	Adolescents		Adults	
		Photopic Illuminance (Mean ± SD lux)	Predicted CS[a] (Mean ± SD)	Photopic Illuminance (Mean ± SD lux)	Predicted CS[a] (Mean ± SD)
0.3	2700 K	282 ± 51	0.27 ± 0.06	290 ± 39	0.27 ± 0.05
	6500 K	195 ± 35	0.27 ± 0.06	213 ± 49	0.29 ± 0.08
0.5	2700 K	747 ± 102	0.45 ± 0.12	773 ± 116	0.45 ± 0.13
	6500 K	467 ± 65	0.44 ± 0.11	536 ± 84	0.47 ± 0.14
0.07	2700 K	53 ± 9	0.06 ± 0.01	56 ± 13	0.07 ± 0.01
	6500 K	32 ± 4	0.06 ± 0.01	31 ± 5	0.05 ± 0.01
0.14	2700 K	100 ± 26	0.11 ± 0.03	109 ± 17	0.12 ± 0.02
	6500 K	62 ± 5	0.11 ± 0.01	66 ± 10	0.11 ± 0.02

Note: (a) The predicted CS values are based on a 1-h exposure.

2.4. Protocol

All participants for each study night were from the same age group and experienced the same experimental condition in a single laboratory. They arrived at the laboratory by 22:30 h and remained in dim light (< 5 lux at the eye) for 30 min, followed by a 3-h exposure to one of the 8 lighting interventions (i.e., 2 spectra × 4 target CS levels). In order to counter a potential subject-expectancy effect, no information concerning the pre-determined, counter-balanced order of experimental conditions was provided to the participants, although subjective assessments were not conducted to ascertain whether the participants could differentiate between the 2 spectra. Over the course of each study night, 7 saliva samples were collected from each participant; the first sample was taken immediately before commencement of the lighting condition after a 30-min dim light exposure, and 6 additional samples were taken thereafter at 30-min intervals (Figure 4). After the final saliva sample was collected at 02:00 h, the participants were released to go home.

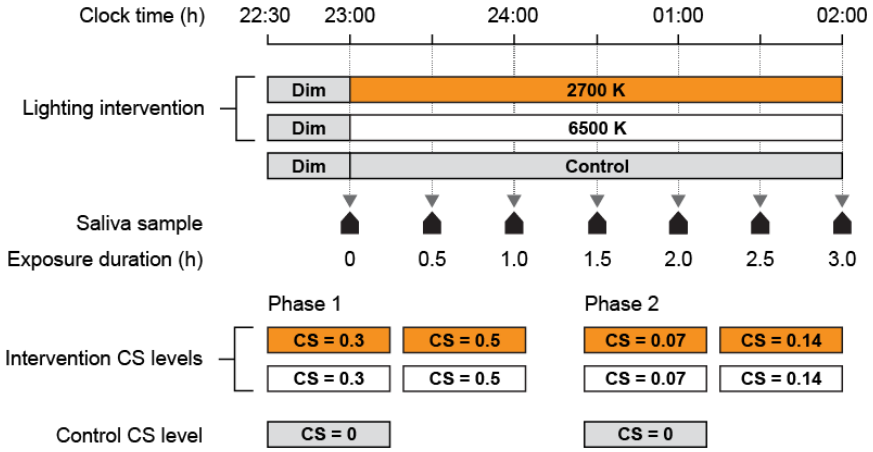

Figure 4. The study protocol. Participants arrived in the laboratory and were held in dim light (< 5 lux at the eye) until the first saliva sample was obtained at 23:00 h. After the first saliva sample was obtained, the desktop luminaires were turned on and 6 additional saliva samples were collected at 30-min intervals.

During the experiment, the participants were instructed to avoid blocking the Daysimeter's sensor and to align their line of sight in the direction of the desktop luminaire to ensure minimum variability with respect to the target stimulus. Participants were free to operate their personal electronic devices (i.e., computers, tablets, cell phones, etc.) but were required to perform a similar task (e.g., browse the internet, watch a video or movie, read an e-book, etc.) on all study nights. All displays were covered with orange-tinted media (Roscolux #21 golden amber, Rosco Laboratories, Stamford, CT, USA) that filtered out radiation < 525 nm to prevent participants from receiving additional circadian-effective stimulus from their self-luminous devices. Periodic visual monitoring was carried out to ensure compliance with the experimental protocol and confirm that none of the participants closed their eyes.

Saliva samples were collected using the Salivette system (Sarstedt, Nümbrecht, DE), wherein the participant chews on a plain cotton cylinder, which is then placed in a test tube and centrifuged for 5 min at 1000 g. Each saliva sample was immediately frozen (-20° C). The frozen samples for each participant were assayed in a single batch using melatonin radioimmunoassay kits

(Direct Melatonin RIA, ALPCO, Salem, NH, USA). The reported sensitivity of the saliva sample assay was 1 pg/ml and the intra- and inter-assay coefficients of variability were 11% and 14%, respectively.

2.5. Data analysis

Melatonin suppression for each condition was determined by comparing melatonin levels collected during the dim light condition (the experimental control) to those collected at the corresponding time on each lighting intervention night. For each study night, melatonin concentrations at the 0.5–3.0 h exposure durations (n = 6) were first normalized to the value for the first sample taken at 23:00 h (see Figure 4), and the melatonin suppression at each of those times was then calculated using the following formula:

$$Percent\ suppression = 1 - \left(\frac{Mn}{Md}\right) \qquad (3)$$

where M_n is the normalized melatonin concentration at each time on respective intervention nights and M_d is the normalized melatonin concentration at each time on the dim light control night.

It is noteworthy that the absolute melatonin levels measured prior to the light exposure, following the initial 30-min dim light adaptation period (22:30–23:00 h), did not significantly differ between the 2 age groups under all of the experimental conditions ($p > 0.05$) (Table 3).

Table 3. Absolute melatonin levels (pg/ml) after 30 min of dark adaptation for each experimental condition. Melatonin levels were not significantly different ($p > 0.05$) between the 2 age groups on any of the study nights.

Age group/ melatonin level	Dim light	Target CS = 0.3		Target CS = 0.5		Dim light	Target CS = 0.07		Target CS = 0.14	
		2700 K	6500 K	2700 K	6500 K		2700 K	6500 K	2700 K	6500 K
Adolescent absolute melatonin level (pg/ml)	11.4	11.1	10.7	11.3	11.6	14.9	13.9	14.1	14.3	15.3
Adult absolute melatonin level (pg/ml)	13.2	13.9	14.2	14.6	13.6	11.5	11.3	9.8	9.3	8.7
Significance *(p)*	0.528	0.406	0.212	0.245	0.530	0.377	0.438	0.180	0.137	0.057

The data were analyzed using linear mixed effects model analysis of variance (ANOVA), with exposure duration, target CS, and spectrum as within factors. Participant age group (i.e., adolescents versus adults) was assigned as a between factor. Further evaluation for main effects and interactions was performed using *post hoc* 2-tailed, Student's *t*-tests. Bonferroni corrections were applied as needed. In some instances, effects were also evaluated using *post hoc* 1-sample *t*-tests. The results of the ANOVA and all t-tests were considered to be statistically significant if the *p* value was < 0.05.

3. Results

The ANOVA revealed a significant main effect of target CS level ($F_{3,159} = 91.8$, $p < 0.001$) and exposure duration ($F_{5,1185} = 92.5$, $p < 0.001$), indicating that higher CS levels and longer exposure durations suppressed melatonin to a greater degree during participants' biological night (Figures 5 and 6). *Post hoc* 2-tailed, Student's *t*-tests of the main effect of exposure duration showed that melatonin suppression after a 3-h light exposure (mean ± SD = 29.5 ± 34.1%) was significantly greater ($p < 0.05$) than after 0.5-hr (10.9 ± 17.7%), 1-h (18.4 ± 23.9%), and 1.5-h (20.8 ± 28.1%) light exposures. Differences in mean ± SD melatonin suppression after 2-h (22.7 ± 30.5%) and 2.5-h (26.6 ± 32.1%) light exposures were not significantly different ($p > 0.05$) compared to the 3-h exposure. Furthermore, there was a statistically significant interaction between the effects of CS level and exposure duration on melatonin suppression ($F_{15,1185} = 13.1$, $p < 0.001$), as is evident from the differing gradients for the 4 curves shown in Figure 7. At lower CS levels, longer exposure durations are required for significant melatonin suppression, whereas significant suppression is observed within 30 min at higher CS levels (Appendix).

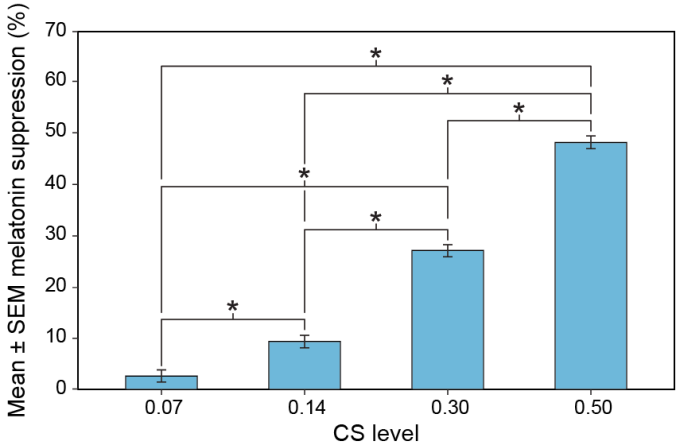

Figure 5. The significant main effect of CS level. The asterisks represent $p < 0.05$.

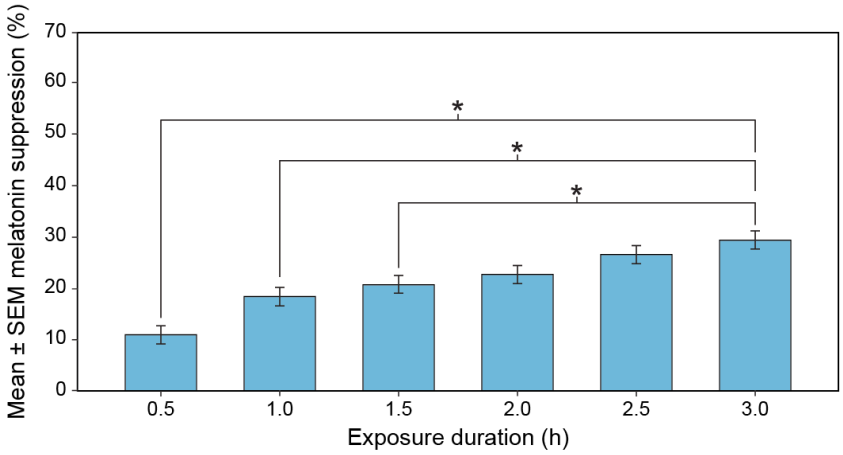

Figure 6. The significant main effect of exposure duration. The asterisks represent $p < 0.05$.

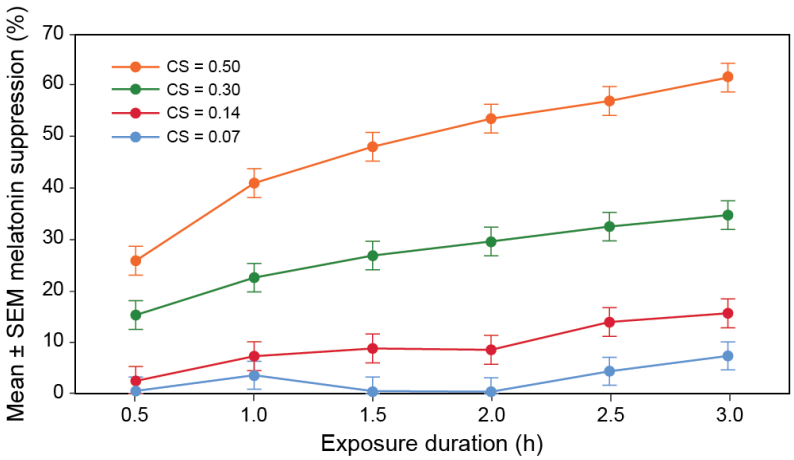

Figure 7. The significant interaction between exposure duration and target CS level ($p < 0.001$).

Exploring this interaction further, *post-hoc* 1-sample t-tests revealed that melatonin suppression at the lowest target CS level of 0.07 was significantly greater than zero only following a 3-h exposure ($t_{67} = 2.22$, $p < 0.05$). For the target CS levels of 0.14, 0.30, and 0.50, melatonin suppression was significantly greater than zero at exposure durations of 1 h ($t_{63} = 4.04$, $p < 0.05$), 0.5 h ($t_{62} = 7.14$, $p < 0.05$), and 0.5 h ($t_{61} = 14.02$, $p < 0.05$), respectively. Given that melatonin assay variability is close to 10%, however, it could be argued that any suppression below 10% would be within that potential measurement error.

To address this matter, we sought to determine whether melatonin suppression at the various target CS levels and exposure durations was significantly greater than 10%. P*ost-hoc* 1-sample *t*-tests revealed that melatonin suppression was significantly greater than 10% for all exposure durations at the 2 high target CS levels (0.30 and 0.50) and only following a 3-h exposure at the lower target CS level of 0.14. At the lowest target CS level of 0.07, melatonin suppression was not significantly greater than 10% at any of the exposure durations (Table 4).

The ANOVA also revealed a significant main effect of spectrum ($F_{1,39} = 8.3$, $p < 0.01$) in which mean melatonin suppression was significantly greater across all experimental conditions combined following exposure to the 6500 K light (mean ± SD = 24.7 ± 27.9%) compared to the 2700 K light (mean ± SD = 18.4 ± 29.5%) (Figure 8).

For the adolescent participants, the mean ± SD melatonin suppression over the entire 3-h session was 18.9 ± 28.2% after exposure to the 2700 K source and 24.6 ± 25.8% after exposure to the 6500 K source. For the adult participants, the mean ± SD melatonin suppression after 3 h was 17.9 ± 30.7% after exposure to the 2700 K source and 24.8 ± 29.8% after exposure to the 6500 K source. Consistent with the results from our previous research, [8] the ANOVA did not reveal a significant interaction between spectrum and exposure duration on melatonin suppression ($F_{5,1185} = 1.99$, $p = 0.08$ [Figure 9]), suggesting that spectral sensitivity of the participants from both age groups did not change during the course of the experimental sessions.

The predicted between-groups, main effect of participant age (Figure 10) on nocturnal melatonin suppression was not statistically significant ($F_{1,39} = 0.19$, $p = 0.67$), nor were any 2-way interactions between the within-subjects variables ($p > 0.05$).

Table 4. Results from *post-hoc* 1-sample t-tests reported for the 4 targeted CS levels and 6 exposure durations. The results shown in bold represent values that are significantly ($p < 0.05$) greater than 10% suppression.

Target CS	Exposure duration (h)	Mean suppression (%)	Analytical Results
0.07	0.5	0.5	$t_{67} = -5.7$; $p = 0.000$
	1.0	3.7	$t_{67} = -3.3$; $p = 0.002$
	1.5	1.5	$t_{67} = -3.1$; $p = 0.003$
	2.0	0.7	$t_{67} = -3.2$; $p = 0.002$
	2.5	4.5	$t_{67} = -1.7$; $p = 0.103$
	3.0	7.0	$t_{67} = -1.0$; $p = 0.335$
0.14	0.5	2.6	$t_{63} = -4.6$; $p = 0.000$
	1.0	8.0	$t_{63} = -1.0$; $p = 0.307$
	1.5	9.8	$t_{63} = -0.1$; $p = 0.942$
	2.0	9.7	$t_{63} = -0.1$; $p = 0.902$
	2.5	14.9	$t_{63} = 1.6$; $p = 0.120$
	3.0	16.8	**$t_{63} = 2.0$; $p = 0.046$**
0.30	0.5	15.4	**$t_{62} = 2.5$; $p = 0.016$**
	1.0	22.6	**$t_{62} = 4.2$; $p = 0.000$**
	1.5	26.9	**$t_{62} = 6.2$; $p = 0.000$**
	2.0	29.7	**$t_{62} = 6.5$; $p = 0.000$**
	2.5	32.5	**$t_{62} = 6.7$; $p = 0.000$**
	3.0	34.8	**$t_{62} = 6.2$; $p = 0.000$**
0.50	0.5	25.9	**$t_{61} = 9.0$; $p = 0.000$**
	1.0	41.0	**$t_{61} = 12.5$; $p = 0.000$**
	1.5	48.1	**$t_{61} = 15.6$; $p = 0.000$**
	2.0	53.5	**$t_{61} = 16.8$; $p = 0.000$**
	2.5	56.9	**$t_{61} = 17.4$; $p = 0.000$**
	3.0	61.5	**$t_{61} = 18.3$; $p = 0.000$**

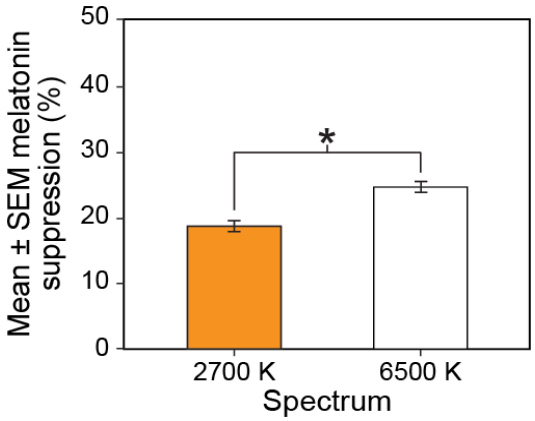

Figure 8. The significant main effect of spectrum for both age groups combined. The asterisk represents $p < 0.05$.

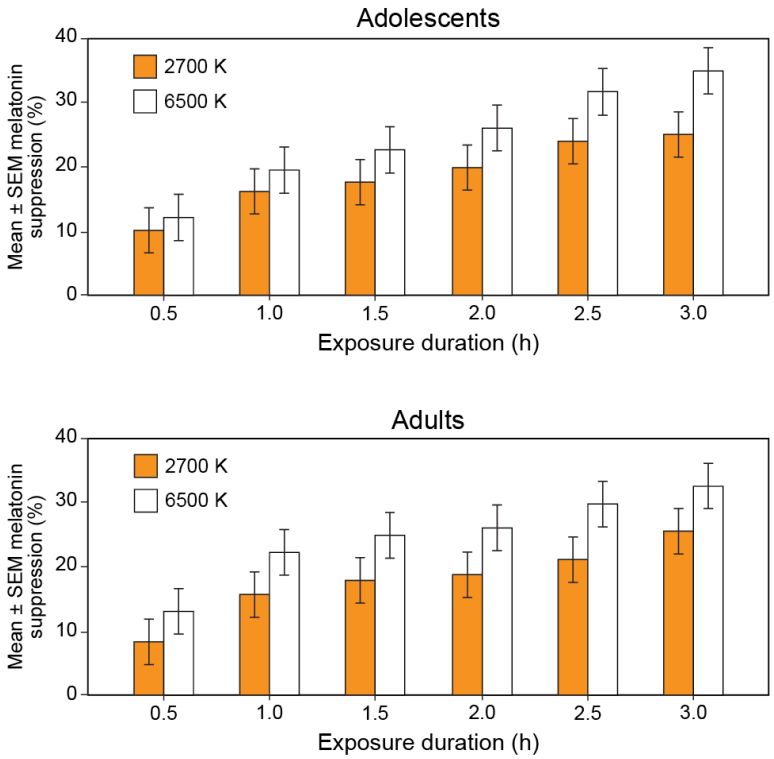

Figure 9. Interaction between exposure duration and spectrum for adolescents (above) and adults (below).

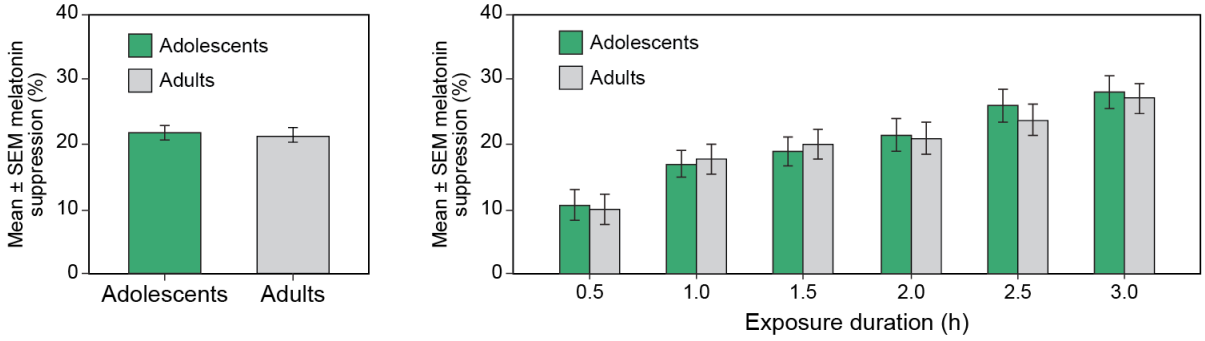

Figure 10. Main effect of participant age on melatonin suppression for all exposure durations combined (left) and by exposure duration (right).

Finally, *post hoc* 1-sample *t*-tests showed that melatonin suppression for both age groups combined was significantly different from zero ($p < 0.05$) following a 1-h exposure to the lighting interventions with target CS levels of 0.50 ($t_{61} = 61.5$; $p < 0.001$), 0.30 ($t_{62} = 7.6$; $p < 0.001$), and 0.14 ($t_{63} = 4.0$; $p < 0.001$). Mean melatonin suppression for both age groups combined (Table 5) was not significantly different from zero following a 1-h exposure to the lowest target CS level of 0.07 ($t_{67} = 1.9$; $p = 0.06$). For the target CS level of 0.07, the mean ± SD melatonin suppression after a 1-h exposure was 2.5 ± 16.1% for the 2700 K source and 4.9 ± 16.1% for the 6500 K source. For the target CS level of 0.14, the mean ± SD melatonin suppression after a 1-h exposure was 7.4 ± 15.0% for the 2700 K source and 8.6 ± 16.8% for the 6500 K source. For the target CS level of 0.30, the mean ± SD melatonin suppression after a 1-h

exposure was 17.0 ± 28.0% for the 2700 K source and 28.1 ± 17.5% for the 6500 K source. For the target CS level of 0.50, the mean ± SD melatonin suppression after a 1-h exposure was 38.7 ± 21.8% for the 2700 K source and 43.2 ± 17.2% for the 6500 K source.

Assessing the ability of the CS model to predict suppression from the spectrometer data (see Table 2), the *post hoc* 1-sample *t*-tests showed that the mean melatonin suppression for both age groups (combined) and spectra was not significantly different from the predicted CS at all light levels (see Table 5). Furthermore, assessing the CS model's ability to predict CS values based on the Daysimeter data (see Table 1), the *post hoc* 1-sample *t*-tests showed that the mean melatonin suppression for both age groups (combined) and spectra was significantly different from the predicted CS at all light levels (see Table 5). These results are most likely attributable to the fact that the Daysimeters were positioned on the participants' foreheads above eye level, whereas the spectroradiometric measurements were taken at eye level. The Daysimeters therefore might have overestimated the amount of light at the eye because the sensor was pointing directly to the light source, while the spectroradiometric measurements were performed with the sensor aligned with participants' line of sight.

Table 5. *Post-hoc* 1-sample *t*-test results assessing accuracy of CS predictions for both age groups combined, generated using Daysimeter and spectrometer data for 2700 K and 6500 K sources.

Prediction method	Target CS	Mean suppression after 1 h (%)	Predicted CS after 1 h	Analysis
2700 K source				
Spectrometer	0.07	2.5	0.06	$t_{33} = -1.3$; $p = 0.21$
	0.14	7.4	0.11	$t_{33} = -1.4$; $p = 0.17$
	0.30	17.0	0.27	$t_{30} = -2.0$; $p = 0.06$
	0.50	38.7	0.45	$t_{30} = -1.6$; $p = 0.12$
Daysimeter	0.07	2.5	0.09	**$t_{33} = -2.4$; $p = 0.02$**
	0.14	7.4	0.16	**$t_{33} = -3.3$; $p = 0.002$**
	0.30	17.0	0.33	**$t_{30} = -3.2$; $p = 0.003$**
	0.50	38.7	0.52	**$t_{30} = -3.4$; $p = 0.002$**
6500 K source				
Spectrometer	0.07	4.9	0.06	$t_{33} = -0.41$; $p = 0.69$
	0.14	8.6	0.11	$t_{29} = -0.78$; $p = 0.44$
	0.30	28.1	0.28	$t_{31} = -0.03$; $p = 0.97$
	0.50	43.2	0.46	$t_{30} = -0.9$; $p = 0.36$
Daysimeter	0.07	4.9	0.09	$t_{33} = -1.5$; $p = 0.15$
	0.14	8.6	0.16	**$t_{29} = -2.4$; $p = 0.02$**
	0.30	28.1	0.34	$t_{31} = -1.9$; $p = 0.06$
	0.50	43.2	0.51	**$t_{30} = -2.5$; $p = 0.02$**

4. Discussion

The present results show that light's effect on melatonin suppression diminishes with increasing exposure duration for both age groups and both light sources. The overall rate of suppression (i.e., the mean absolute percent suppression per hour of exposure) was 34.8% (1 h), 22.2% (2 h), and 17.3% (3 h), again highlighting the human circadian system's non-linear dose-dependent response to photic stimuli. [6-8] The interaction between exposure duration and target CS level also suggests that it takes longer to observe significant melatonin suppression at lower CS levels than at higher CS levels. [34,35]

The American Medical Association recently issued a report recommending the use of outdoor lighting with CCTs no greater than 3000 K. [36] As precise measurements of light exposures in the field are essentially lacking at present, however, light's beneficial or detrimental effects cannot be clearly articulated. One of the goals of the present study was to investigate threshold levels for acute melatonin suppression and thereby provide empirical data as a basis for the discussion of light's impact on the circadian system. Table A.1 shows the predicted threshold and half-maximum saturation photopic illuminance levels for each exposure duration and spectrum, and Figure A.1 shows the best fits used to estimate those threshold values (see Appendix). As shown in Table A.1, the light levels typically recommended by the Illuminating Engineering Society of North America for outdoor environments at night (18 lux on the horizontal plane) are below the threshold for melatonin suppression observed by the present study, even after a 3-h exposure. [37] Computer displays and portable electronic devices, on the other hand, could potentially suppress melatonin at night, as has been shown by our previous studies. [22,38] The proposed melatonin suppression threshold of 30 lux for 30 min suggested by Rea and colleagues in various publications [39-42] appears to be acceptable, if one wants to be conservative. Note, however, that the threshold used in this study was a CS level of 0.1 (equivalent to 10% melatonin suppression after a 1-h exposure) because anything below 10% is within assay measurement error. It should also be noted that while acute melatonin suppression and phase shifting are likely to have the same spectral sensitivity (i.e., they are more sensitive to short wavelength light), their absolute sensitivities and temporal characteristics may not be the same. Therefore, caution should be taken when extrapolating these acute melatonin suppression results to other outcomes of the circadian system.

The present results do not corroborate the study's hypotheses that adolescents exhibit greater circadian sensitivity to short-wavelength radiation compared to adults, which is inconsistent with our previous work [8] showing that melatonin suppression was significantly greater after exposure to a 5600 K intervention (43%) compared to a 2700 K intervention (29%) for adolescents only. A similar trend was reported by Gabel *et al.*, [43] who showed that melatonin suppression after exposures to matched levels (250 lux) of warm (2800 K) and blue-enriched (9000 K) light was similar for adults (n = 12; mean ± SEM age = 63.6 ± 1.3 years), but suppression was more pronounced for young adults (n = 26; mean ± SEM age =25.0 ± 0.6 years) after exposure to the blue-enriched light. It is important, however, to view the present results in the context of the adult participants' mean age (mean ± SD age of 42.4 ± 10.9 years), which was somewhat younger than the adult participants in the Nagare *et al.* study (mean ± SD age of 46 ± 5.2 years) [8]

and considerably younger than those in the Gabel *et al.* study (mean age ± SEM age of 63.58 ± 1.27 years). [43]

Furthermore, it is noteworthy that not all previous studies employing melatonin as a circadian biomarker have reported differential circadian sensitivity in respect to age. [44-46] A recent study by Najjar *et al.* [47] investigating a series of non-visual responses to 60-min duration, monochromatic light exposures showed that melatonin suppression was not significantly different between older participants (mean ± SEM age of 59.4 ± 0.99 years) and younger participants (mean ± SEM age of 25.8 ± 0.73 years) when matched for photic stimulus at 480 nm. Thus, it is clear that additional research is needed to better understand how age-related physiological changes affect the light sensitivity of the human circadian system.

5. References

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Appendix: Predicted Threshold and Half-maximum Saturation Photopic Illuminance Levels, by Light Source and Age Group

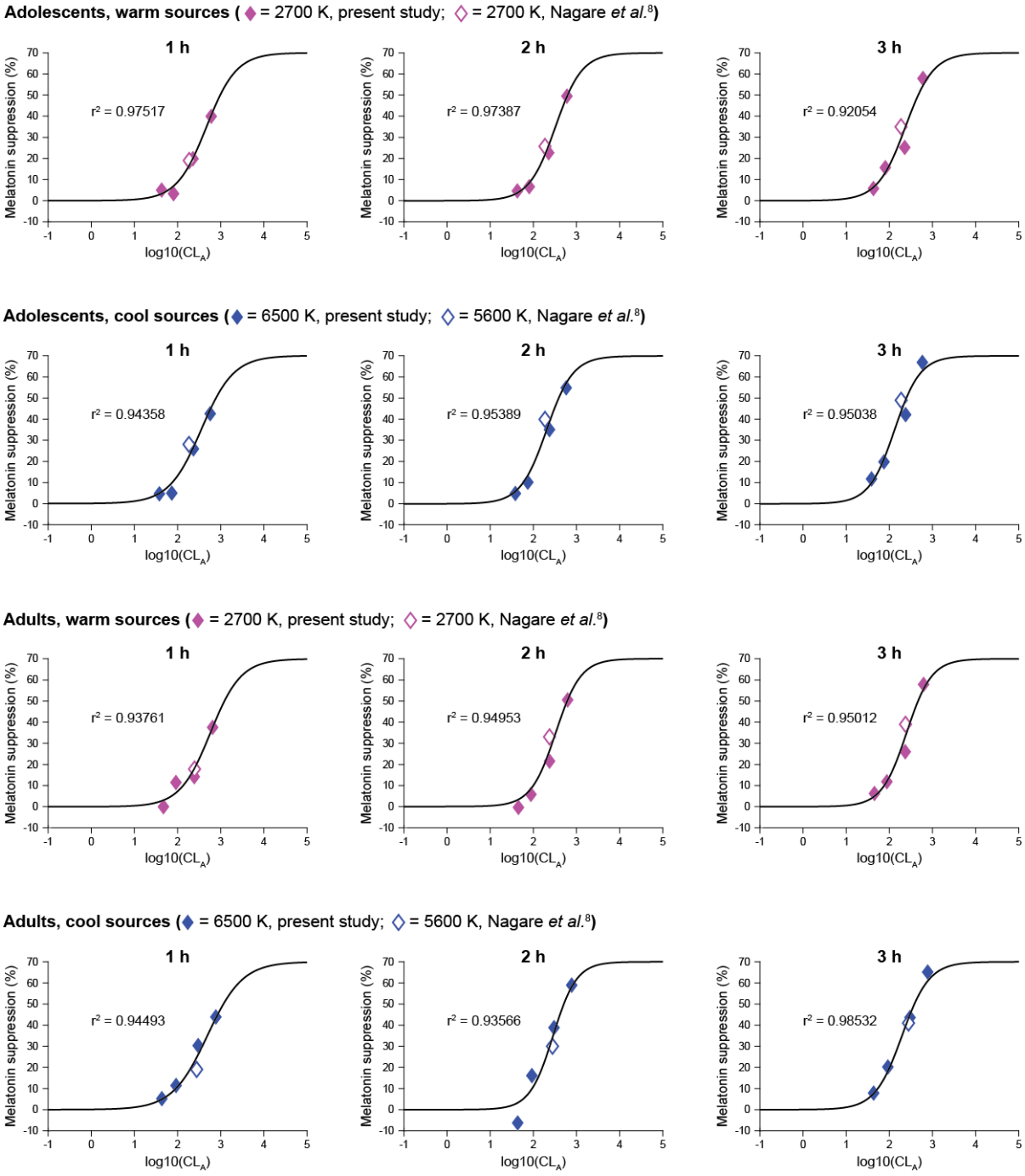

Figure A.1. The least squares method, a 3-parameter logistic function [12] that converts CL_A to CS, was used to best-fit the melatonin suppression data at each hourly exposure duration, by age group and light source. The warm sources include the 2700 K source from the present study (solid polygons) and a similar 2700 K source from a previous white light study (hollow polygons). [8] The cool sources include the 6500 K source from the present study (solid polygons) and a similar 5600 K source from the same, previous white light study (hollow polygons). [8] The threshold (CS= 0.1) and half-saturation photopic illuminance levels, derived using the respective best-fit plots, are summarized in Table A.1.

Table A.1. Threshold (CS= 0.1) and half-maximum saturation (CS = 0.35) photopic illuminance levels for each light source and age group by hourly exposure duration derived from the best-fit plots in Figure A.1.

Exposure duration (h)	Threshold photopic illuminance (lux)				Half-maximum saturation photopic illuminance (lux)			
	2700 K		6500 K		2700 K		6500 K	
	Adolescents	Adults	Adolescents	Adults	Adolescents	Adults	Adolescents	Adults
1	154	185	71	85	582	713	294	402
2	125	138	53	74	406	411	169	238
3	86	104	36	49	294	312	109	163