High sensitivity and interindividual variability in the response of the human circadian system to evening light

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Published online May 28, 2019.

Significance

Electric lighting has fundamentally altered how the human circadian clock synchronizes to the day/night cycle. Exposure to light after dusk is pervasive in the modern world. We examined group-level sensitivity of the circadian system to evening light and the degree to which sensitivity varies between individuals. We found that, on average, humans are highly sensitive to evening light. Specifically, 50% suppression of melatonin occurred at <30 lux, which is comparable to or lower than typical indoor lighting used at night, as well as light produced by electronic devices. Significantly, there was a >50-fold difference in sensitivity to evening light across individuals. Intermittidividual differences in light sensitivity may explain differential vulnerability to circadian disruption and subsequent impact on human health.

Author contributions: S.W.L. and S.W.C. designed research; P.V., A.C.B., and E.M.M. performed research; A.J.K.P., P.V., A.C.B., E.M.M., S.W.L., and S.W.C. analyzed data; and A.J.K.P., P.V., A.C.B., E.M.M., C.A., S.M.W.R., S.W.L., and S.W.C. wrote the paper.

Conflict of interest statement: C.A. has received a research award/prize from Sanofi-Aventis; contract research support from VicRoads, Rio Tinto Coal Australia, National Transport Commission, and Tontine/Pacific Brands; lecturing fees from Brown Medical School/Rhode Island Hospital, Amed, Healthmed, and TEVA Pharmaceuticals; and reimbursements for conference travel expenses from Philips Healthcare. C.A. has served as a consultant to the Rail, Bus, and Tram Union; the Transport Accident Commission; the National Transportation Committee; and Melius Consulting. C.A. has also served as an expert witness and/or consultant in relation to fatigue and drowsy driving. C.A. is a Theme Leader in the Cooperative Research Centre for Alertness, Safety, and Productivity. S.W.R. has served as a consultant through his institution to Vanda Pharmaceuticals, Philips Respironics, and Teva Pharma Australia and has, through his institution, received research grants and/or unrestricted educational grants from Vanda Pharmaceuticals, Takeda Pharmaceuticals North America, Philips Lighting, Philips Respironics, Cephalon, and RedMed Foundation, as well as reimbursements for conference travel expenses from Vanda Pharmaceuticals. S.M.W.R. serves as a Program Leader and consultant to the Cooperative Research Centre for Alertness, Safety, and Productivity. S.W.L. has had a number of commercial interests in the last 36 mo (2016-2019). None are directly related to the research reported in this paper but, in the interests of full disclosure, are outlined as follows. S.W.L. has received consulting fees from the Atlanta Falcons, Atlanta Hawks, BHP Billiton, Noble Insights, Slinghot Insights, and Team C Racing; honoraria and/or paid travel from BHP Billiton, DNV, Emory University, IES, Ineos, SLTBR, Solemaria, and Teagasc; has current consulting contracts with Akil Interactive, Apex 2100 Ltd., Consumer Sleep Solutions, Headwaters Inc., Hintsa Performance AG, Light Cognitive, Lighting Science Group Corporation, Mental Workout, PlanLED, Six Senses, Stantec, and Wyile Integrated Science and Engineering; has received unrestricted equipment gifts from Biometrics Corporation and F. Lux Software LLC and royalties from Oxford University Press; and has served as a paid expert in legal proceedings related to light, sleep, and health. He holds a patent through Harvard University and Brigham and Women’s Hospital for “Systems and Methods for Determining and/or Controlling Sleep Quality.” This article is a PNAS Direct Submission. D.B.B. is a guest editor invited by the Editorial Board. This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

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www.pnas.org/cgi/doi/10.1073/pnas.1901824116

Before the invention of electric lighting, humans were primarily exposed to intense (>300 lux) or dim (<30 lux) environmental light—stimuli at extreme ends of the circadian system’s dose-response curve to light. Today, humans spend hours per day exposed to intermediate light intensities (30–300 lux), particularly in the evening. Interindividual differences in sensitivity to evening light in this intensity range could therefore represent a source of vulnerability to circadian disruption by modern lighting. We characterized individual-level dose-response curves to light-induced melatonin suppression using a within-subjects protocol. Fifty-five participants (aged 18–30) were exposed to a dim control (<1 lux) and a range of experimental light levels (10–2000 lux for 5 h) in the evening. Melatonin suppression was determined for each light level, and the effective dose for 50% suppression (ED50) was computed at individual and group levels. The group-level fitted ED50 was 24.60 lux, indicating that the circadian system is highly sensitive to evening light at typical indoor levels. Light intensities of 10, 30, and 50 lux resulted in later apparent melatonin onsets by 22, 77, and 109 min, respectively. Individual-level ED50 values ranged by over an order of magnitude (6 lux in the most sensitive individual, 350 lux in the least sensitive individual), with a 26% coefficient of variation. These findings demonstrate that the same evening-light environment is registered by the circadian system very differently between individuals. This interindividual variability may be an important factor for determining the circadian clock’s role in human health and disease.

circadian rhythms | light sensitivity | circadian disruption | melatonin suppression | evening light

It was once thought that the human circadian system was relatively insensitive to light, with synchronization of our clocks to the 24-h day achieved primarily through nonphotic cues, such as social interactions (1). It is now widely accepted that light is the primary synchronizing stimulus for the human circadian system (2–4). In a landmark study, Zeitzer et al. (5) measured responses of the human circadian system to nocturnal light as a function of the light’s intensity. The resultant dose–response curves demonstrated that half of the maximum-phase resetting and melatonin-suppression responses are achieved at light levels of only ~100 lux. Nearly all of the change from no responsiveness to maximal responsiveness occurred between 30 and 300 lux.

Before the invention of electric light, humans would have spent nearly all of their time exposed to light intensities at the minimal and maximal ends of the dose–response curve: natural daylight (>300 lux) or dim sources of light after sunset (<30 lux), including moonlight and small fires (6–11). Today’s light environment differs considerably in the amount of time spent at intermediate light intensities (30–300 lux); i.e., in the steepest region of the dose–response curve. Exposure to electric light after sunset in this range is extremely common in industrialized countries (9, 12–14), and the increasing use of light-emitting devices is a further source of evening light that is potentially disruptive to the circadian system (15, 16).
Interindividual differences in light sensitivity are most likely to be impactful where the dose–response curve to light is steepest: at intermediate light levels. To date, interindividual differences in the dose–response curve to light have not been systematically studied. The dose–response curves measured by Zeitzer et al. (5) were group averages, with each of the 21 data points obtained from a single light exposure in a different participant. Results of other studies suggest that interindividual differences in the dose–response curve to light could be present (17–23), including one study of dose–response curves to light in six individuals (24). Gooley et al. (20) found a range of melatonin-suppression responses (29–93%) to the same <200-lux light stimulus. The present study examined group- and individual-level dose–response curves for the human circadian system to evening light by systematically varying light exposures within individuals for a total of 351 nights of data across 55 individuals. Using highly controlled light-exposure techniques, we found that humans are more sensitive to evening light than previously thought and that there is over an order of magnitude difference in sensitivity across individuals.

Results
A total of 27 healthy men [mean ± SD; body mass index (BMI) of 23.07 ± 2.43 kg/m²] and 29 healthy women (mean ± SD; BMI of 23.46 ± 2.23 kg/m²), aged 18–30 y (mean ± SD; age of 20.8 ± 2.6 y), completed a 6- to 7-wk protocol with structured sleep maintained throughout. The first week ended with a baseline dim-light (<1 lux) control condition, followed by weekly white-light exposures in a group-randomized order, counterbalanced for sex. Light-exposure levels included 10, 30, 50, 100, 200, 400, and 2,000 lux. Light exposures began 4 h before habitual bedtime and ended 1 h after habitual bedtime. Hourly saliva samples were collected and assayed for melatonin by radioimmunoassay. Melatonin suppression was calculated for each light condition as a percentage of baseline melatonin. To control for the circadian timing of light exposure, melatonin suppression was calculated from the baseline dim-light melatonin onset (DLMO) onward for each individual, using either all data after DLMO (overall) or for each of the first 3 h after DLMO (hours 1–3). Baseline DLMO was able to be determined for 55 of 56 participants, resulting in 1 exclusion.

High Group-Level Sensitivity to Evening Light. Percentage melatonin suppression was measured for each individual at each time point under each light condition (n = 55 participants after one exclusion, 351 nights of data, and 2,692 valid melatonin samples). Group-level logistic dose–response curves were fit to pooled individual data points for hour 1 (n = 55; 281 suppression values), hour 2 (n = 50; 254 suppression values), and hour 3 (n = 36; 184 suppression values) of light exposure, following DLMO, as well as overall (all data points after DLMO; 281 suppression values). There were fewer data points in later hours due to individuals with later DLMOs relative to bedtime having shorter durations of post-DLMO light. The ED₅₀ values were 24.60 lux (95% CI: 21.33–28.37 lux) for overall, 13.47 lux (95% CI: 11.77–15.42 lux) for hour 1, 19.38 lux (95% CI: 16.61–22.63 lux) for hour 2, and 38.89 lux (95% CI: 32.33–46.77 lux) for hour 3 (Fig. 1). These ED₅₀ values, measured under ecologically relevant light intensities, reflect a high sensitivity of the circadian system to evening light. Melatonin suppression was greatest immediately after DLMO, at a time when exposure to electric lighting is very common.

High Individual Variability in Evening Light Sensitivity. Individual-level dose–response curves (Fig. 1) showed high interindividual variability in responsiveness to evening light. Accurate estimates of ED₅₀ (defined as 95% CI <1 log-unit) were obtained for 42 participants for overall, 41 for hour 1, 37 for hour 2, and 29 for hour 3. For the overall fits, individual ED₅₀ values ranged from 6.0 lux (95% CI: 2.1–16.6 lux) to 349.8 lux (95% CI: 187.7–651.9 lux), with a coefficient of variation of 26.0% for the log₁₀ED₅₀ values (overall data are also expressed in terms of melanopic lux in SI Appendix, Fig. S1). For hourly fits, the individual ED₅₀ values ranged from 2.9 to 303.0 lux for hour 1, 5.2–424.2 lux for hour 2, and 10.9–153.5 lux for hour 3. The ED₅₀ coefficients of variation were 34.7% for hour 1, 33.5% for hour 2, and 21.6% for hour 3. We note that the ED₅₀ range was lower for hour 3 since the least-sensitive individual contributed data only for hours 1–2 due to a late DLMO relative to their habitual bedtime. The ED₅₀ values displayed potentially bimodal distributions (Fig. 1) for overall (kurtosis = 12.8), hour 1 (kurtosis = 15.7), and hour 2 (kurtosis = 18.7), but not for hour 3 (kurtosis = 2.1). Potential bimodality could not be accounted for by any factors we examined [sex, age, habitual bedtime, DLMO, phase angle, morningness–eveningness questionnaire (MEQ), experimental order, or season].

Interindividual differences in the dose–response curve to light were considerable. As illustrated by the low- and high-sensitivity participants in Fig. 2, the individual with low sensitivity responded similarly to 400 lux (51.4% melatonin suppression) as the individual with high sensitivity did to only 10 lux (52.7% melatonin suppression). These two individuals would therefore respond very differently to the same home-lighting environment.

Contrast analyses for overall ED₅₀-DLMO, and phase angle confirmed no order effects (all P > 0.05). In addition, we found no

![Fig. 1. Melatonin suppression depends on light intensity and time of exposure and exhibits large interindividual differences. Columns correspond to overall data (i.e., all data after DLMO) and hours 1–3 after DLMO. Top shows group-level dose–response curves to light. ED₅₀ values are indicated. Error bars represent mean ± SEM. Gray shaded areas in the top row represent 95% CIs for the fitted logistic curve. Middle shows individual-level dose–response curves. Individual curves (solid lines) and corresponding data points (crosses) are colored from blue (lowest ED₅₀) to red (highest ED₅₀). Individual-level curves are shown only for individuals with a reliable ED₅₀ value (95% CI <1 log-unit). Bottom shows histograms of the individual ED₅₀ values, with accompanying box plot and individual data points colored from blue (lowest ED₅₀) to red (highest ED₅₀).](image-url)
significant relationship between total melatonin production and individual-level overall ED\textsubscript{50} values \((r = -0.18, P = 0.18)\). Nor did we find significant relationships of individual-level overall ED\textsubscript{50} values with either DLMO time \((r = -0.09, P = 0.53)\) or the phase angle between DLMO and habitual bedtime \((r = -0.01, P = 0.94)\), which determined the length of experimental light exposure before DLMO. Furthermore, we found no associations between multiple measures of light-exposure history (mean lux, log mean lux, median lux, hours >100 lux, and hours >500 lux) and individual-level overall ED\textsubscript{50} values \((P > 0.15; SI\ Appendix; Table S1)\). These null findings confirm that the individual differences in ED\textsubscript{50} values are not simply due to differing lengths of exposure to light before DLMO or due to differing light-exposure histories.

**Melatonin Onset Under Light Depends on Individual-Level Sensitivity.** The rise of melatonin signals the beginning of the biological night and promotes sleep onset, but light can affect the timing of this rise. We examined how different levels of evening-light exposure affect the timing of “apparent” melatonin onset, compared with the timing of baseline DLMO (i.e., melatonin onset assessed under truly dim light of <1 lux). A dose–response relationship was observed, with apparent melatonin onset occurring progressively later for progressively higher light levels (Fig. 3). In 10 lux, apparent melatonin onset occurred an average of 22 ± 43 min later than DLMO measured under <1 lux conditions \((P < 0.001)\) and ≥29 min later in 50% of individuals. At 30 lux, apparent melatonin onset occurred an average of 77 ± 56 min later than DLMO \((P < 0.00001)\) and ≥68 min later in 50% of individuals. At 50 lux, apparent melatonin onset occurred an average of 109 ± 46 min later than DLMO \((P < 10^{-10})\) and ≥106 min later in 50% of individuals. These findings indicate that even relatively dim light has substantial effects on the apparent timing of melatonin onset, compared with assessment under truly dim-light (<1 lux) conditions.

The change in an individual’s apparent timing of melatonin onset relative to baseline DLMO was associated with their ED\textsubscript{50}. Specifically, an individual’s ED\textsubscript{50} level was significantly associated with the estimated level of light required to move the apparent melatonin onset 1 h later than baseline DLMO \((r^2 = 0.53, P < 0.0001)\).

**Discussion** Our data demonstrate that the human circadian system is highly responsive to dim evening light. At the group-average level, we observed >50% melatonin suppression at 30 lux. Furthermore, we found substantial interindividual differences in sensitivity to light by generating individual-level dose–response curves to light using a highly controlled within-subjects protocol. The four most-sensitive participants exhibited >50% melatonin suppression in response to dim reading light (10 lux), whereas the least-sensitive participant did not achieve this level of suppression until exposed to bright indoor light of 400 lux.

Our study systematically examined interindividual differences in light sensitivity of the human circadian system in a large sample. The interindividual differences we observed in light sensitivity may underlie interindividual differences in sleep and circadian timing that are known to arise with access to electric light (8, 25, 26), in addition to previously identified physiological factors, including intrinsic period of the circadian clock and sleep homeostatic parameters (27–30). We note that differences in light sensitivity might also have been an important confound in classical circadian studies. In early studies of human circadian rhythms that did not control for exposure to indoor room light, circadian periods exhibited a 30% coefficient of variation \((1)\), similar to the 26% coefficient of variation we observe in ED\textsubscript{50} values. In subsequent studies conducted under controlled, dim-light conditions, the coefficient of variation in the human circadian period reduced to <0.6% \((4)\), showing that the human circadian period distribution was in fact similar to that seen in nonhuman animals studied in dark conditions. Thus, the previously high coefficient of variation reported in human period estimates may have been a consequence of the variability in human circadian light sensitivity, which manifested as differences in estimated period due to uncontrolled indoor lighting.

Abnormalities in the sensitivity of the human circadian system to light may contribute to sleep and circadian disruption. Individuals with very high sensitivity (low ED\textsubscript{50} values) would be particularly vulnerable to sleep and circadian disruption even by dim indoor light. This is consistent with findings of higher light sensitivity in individuals with delayed sleep–wake–phase disorder compared with healthy controls, based either on measurements of melatonin suppression \((31)\) or phase shifting \((22)\). Increased melatonin suppression has also been observed in patients with bipolar disorder \((32–34)\) and seasonal affective disorder (SAD) \((33, 35)\). Patients with SAD may also exhibit seasonal hypersensitivity to light, suggesting that abnormally high or low light sensitivity may be associated with pathology \((35)\). Methods to efficiently phenotype an individual’s light sensitivity \(e.g.,\) by using melatonin suppression or pupillary measures \((36)\) will likely be needed to develop personalized interventions based on an individual’s circadian physiology.

The large interindividual differences observed in our study may be the result of several different factors. We recently showed that interindividual differences in melatonin suppression relate to functional MRI BOLD activation in the suprachiasmatic area \((37)\). However, it is not clear whether this difference is due to differences at the level of the retina, suprachiasmatic nuclei (SCN) function, or other modulating inputs to the SCN. It has been shown that interindividual differences in the sustained pupil responses to light (likely melanopsin-based) relate to differences in midsleep time \((38)\), the diagnosis of SAD \((39)\), and delayed sleep–wake–phase disorder \((22)\). A recent analysis indicates that melatonin suppression may indeed be driven primarily by melanopsin photoreception \((40)\). It is, however, also possible that interindividual differences exist in the processing of light information at the level of the SCN, independent of differences in retinal signaling \(e.g.,\) glutamate or pituitary adenylyl cyclase-activating polypeptide receptor function. Furthermore, it is likely that nonretinal input to the SCN can modulate the response to light. As selective serotonin reuptake inhibitors have been found to modulate light sensitivity \((41)\), interindividual variability in raphe input to the SCN is one highly plausible mechanism for the differences we observe in light sensitivity. Further study will be needed to tease apart the many
potential mechanisms for interindividual differences in circadian light sensitivity.

Light exposures in this study were designed to occur in the evening (beginning before DLMO) and included intensities that are frequently experienced in the home. This seminaturalistic design is different from that used by Zeitzer et al. (5), who studied later light exposures that were timed to mostly overlap with peak melatonin production (typically beginning after DLMO) and reported far lower group-level sensitivity to light than reported here. The differences in experimental design could account for the reported differences in light sensitivity. Specifically, our pre-DLMO light exposures may have suppressed the rise in melatonin while also phase-delaying the circadian clock and therefore the signal for the rise of melatonin. Although we found higher sensitivity than Zeitzer et al. (5), we did observe larger ED50 values (less sensitivity) progressing from hours 1 to 3 after DLMO, as our light exposure more closely approached the timing used by Zeitzer et al. (5). We note, however, that the Zeitzer et al. (5) study included only three data points in the 10- to 50-lux range and only two data points in the 50- to 100-lux range, meaning that their group-level ED50 estimate depended strongly on the light sensitivities of those five participants. Our findings of a general high sensitivity to light are corroborated by studies employing blue-light exposures (42), including a recent study by Vartanian et al. (43), who found that melatonin suppression can be induced even by very dim blue light. These findings indicate that the rise of melatonin, an important signal for the initiation of sleep that inhibits electrical activity of SCN neurons (44), is extremely sensitive to suppression by evening-light exposure.

The importance of the high sensitivity to evening light extends to the implementation of “dim-light” conditions for studies that measure DLMO—the current gold-standard method for assessing circadian phase. Whereas the Zeitzer et al. (5) dose–response curve for nocturnal light exposure indicates negligible (<10%) melatonin suppression at intensities of 50 lux or less, our findings show that evening-light exposure at intensities of 10, 30, and 50 lux beginning 4 h before bedtime (more typical for DLMO procedures) is likely to decrease with age (49).

Fig. 3. The time at which an individual crosses the DLMO threshold has a dose–response relationship with light intensity. Upper shows the percentage of participants who had crossed the 4-pg/mL (DLMO) threshold at a given time after baseline DLMO. Colored curves correspond to different light intensities. Lower shows the relationship between individual-level ED50 values and the estimated level of light required to move the apparent melatonin onset 1 h later than baseline DLMO. The gray dashed line shows a linear regression to the log-log values. Lower, Insets represent three individuals: high sensitivity (blue inset), intermediate sensitivity (purple inset), and low sensitivity (red inset). In each inset, melatonin concentration is plotted with respect to clock time for baseline (black curve) and at 30 lux (gray curve). Horizontal dashed lines indicate the 4-pg/mL threshold. Vertical lines indicate times at which melatonin concentration crossed the 4-pg/mL threshold. Included are all participants with an accurate ED50 estimate (95% CI < 1 log10-unit).
are most commonly exposed to in the evening in industrialized societies. For typical indoor light (~50 lux), we found that some individuals respond as if exposed to bright natural daytime light, whereas other individuals respond in the same way as they do to a very dim light source. Under naturalistic lighting conditions in which humans evolved, large interindividual differences in the response to intermediate light levels would likely have been inconsequential, due to minimal time exposed to this narrow range of light levels. In modern industrialized society, however, exposure to intermediate light levels in the evening is virtually ubiquitous, especially due to the proliferation of hand-held blue-enriched light sources to which the circadian system is particularly sensitive. These interindividual differences in sensitivity to light may therefore be an important and previously unappreciated determinant of the circadian clock’s role in human health and disease.

Methods

All procedures were approved by the Monash University Human Research Ethics Committee before commencement. Participants gave written informed consent and were reimbursed for their time.

Participants. A total of 61 participants were enrolled, of whom 3 were excluded based on actigraphy, and 2 did not complete the study beyond the baseline DLMO. Overall, 56 healthy young Caucasian adults (29 women, 27 men; 20.8 ± 2.6 y of age) completed the study. Participants were free from any medical or psychological conditions, had a BMI of 18–30 kg/m², and were not taking any medications at the time of the study. Participants had not recently traveled across time zones (1 mo per time zone, up to 3 mo) or engaged in shiftwork in the previous 12 mo. Women were naturally cycling (i.e., free from hormonal contraception) and had a regular menstrual cycle of 21–36 d in duration. Participants were healthy sleepers, reporting no subjective problems or previous diagnoses, and having a regular bedtime before 1 AM. A score of 10 or greater on the Epworth Sleepiness Scale was exclusionary, and participants were predominantly intermediate chronotypes (MEQ score of 52.7 ± 9.2). A participant had an average bedtime and waketime of 23:04 (SD = 44 min) and 07:04 (SD = 44 min), respectively. DLMO occurred on average ±2.2 h before participants were instructed to wear prescription glasses at each test session. We did not select participants based on their habitual light-exposure history, nor did we instruct them on light-exposure patterns, other than the strict sleepwake times required for the study. Analysis of light-exposure history is presented in SI Appendix, Table S4.

Protocol. Participants completed a total of either six or seven consecutive weekly in-laboratory test sessions. All light exposures commenced 4 h before bedtime and concluded 1 h after. The first session was a dim-light control (<1 lux), with either five or six subsequent experimental light exposures of varying intensities (10; 30; 50; 100; 200; 400; and 2,000 lux; see SI Appendix, Fig. S2 for sample protocol). Participants were randomly assigned to one of six randomly generated light-exposure orders, with each order counterbalanced for sex: (i) 100; 200; 10; 200; 400; and 2,000 lux; (ii) 10; 200; 10; 20; 400; and 2,000 lux; (iii) 200; 400; 200; 10; 100; and 50 lux; (iv) 50; 20; 500; 100; 200; and 50 lux; (v) 10; 200; 10; 20; 400; and 2,000 lux; (vi) 10; 200; 10; 20; 400; and 2,000 lux. The effective illuminance for human photopigments in the retina across all light-exposure conditions is listed in SI Appendix, Table S3, calculated by using the Irradiance Toolbox (42). Light intensities were measured and tested at least an hour before participants’ arrival at the laboratory and remained stable within and between test sessions.

Melatonin Assays. Saliva samples were collected hourly by using salivettes (Salimetrics, Inc.). Saliva sampling began 4 h before habitual bedtime and ended 1 h after habitual bedtime. This totaled six samples ({−3, −2, −1, 0, +1} h). Participants were not permitted to clean their teeth during the collection period and were instructed to drink after eating to rinse their mouth. Food and drinks were strictly forbidden during the 20 min before a sample being taken, and participants were instructed to not change their posture for at least 20 min before each sample was collected. Samples were centrifuged at 2,500 rpm for 5 min (Centrifuge 5702R, Eppendorf), before being placed in a −35 °C freezer for storage until assay. Samples were radioimmunoassayed in duplicate with the G280 antibody and the [125I]2-iodomelatonin radioligand to determine melatonin concentration (University of Adelaide). The interassay and intraassay coefficients of variation were <17% and 7%, respectively.

Data Analysis. Salivary DLMO was determined by using an absolute threshold of 4 pg/mL. Linear interpolation was used to determine the first threshold-crossing time. DLMO values were obtained for 55 of the 56 participants. One participant was excluded from analyses as their DLMO could not be determined, since they did not exceed the threshold on any of the baseline assays. Melatonin-suppression values were calculated for each participant under each experimental light-exposure condition. Melatonin suppression was calculated by using one of four time intervals: (i) overall: from baseline DLMO to final assay (whichever was earlier of the baseline final assay and experimental final assay); (ii) hour 1: from baseline DLMO to 1 h post-DLMO; (iii) hour 2: from 1 h post-DLMO to 2 h post-DLMO; and (iv) hour 3: from 2 h post-DLMO to 3 h post-DLMO. Linearly interpolated curves were generated for both baseline and experimental light-exposure conditions. Percentage melatonin suppression was the percentage difference in area under the curve between the two interpolated curves within the specified time interval. For the hourly time intervals, we required at least 30 min of overlap where both linearly interpolated curves were defined. Undefined values could occur due to missing assays or due to the final assay occurring within the time interval. Previous work has demonstrated that melatonin-suppression dose-response curves are best described by a four-parameter logistic model (5). This model was fit to our data: % Suppression = a \left( \frac{x - b}{c - d} \right) + e

where y0 is the minimum melatonin-suppression response, a is the difference between the minimum and maximum response, x0 is the ED50, and b is the slope parameter that determines how quickly the curve rises. We made the physiologically reasonable assumptions of 0% suppression at 0 lux and 100% suppression as photopic illuminate approaches infinity (y0 = 0, a = 100).
We thank the research participants for their dedication and cooperation, as well as the SuperMega research staff and students at the Monash University Sleep and Circadian Medicine Laboratory. We give special thanks to Dr. Melissa St. Hilaire for statistical consultation. This work was supported by National Health and Medical Research Council (NHMRC) Grant NHMRC 1064231 (to S.W.C.). P.V. was supported by a NeuroSleep NHMRC Centre of Research Excellence PhD scholarship. E.M.M. and A.C.B. were supported by Australian Government Research Training Program scholarships.

ACKNOWLEDGMENTS.

the dataset, we observed full or near-to-full (>99%) suppression on 61 nights, justifying this assumption. Curves were fit at both the group and individual levels by using the Levenberg-Marquardt residual minimization procedure, and we implemented in Matlab using the inbuilt function nlinfit. Percentage melatonin-suppression values for all experimental conditions were the y variable; logt-transformed photopic illuminance values for all experimental conditions were the x variable. To ensure convergence of the fit, y values were bounded to a minimum of 0.1% and a maximum of 99.9%. Individual-level dose–response curves were assumed to be monotonically increasing, but we allowed up to a 20% temporary error and a between-individual variability (i.e., each melatonin-suppression value on the dose–response curve could be up to 20% less than suppression values at any lower photopic illuminances). Values that did not satisfy this tolerance were excluded (4.3% of suppression values for overall, 4.1% for hour 1, 3.8% for hour 2, and 2.6% for hour 3). We required a minimum of three data points (i.e., valid melatonin-suppression values at three exponential light levels) to perform individual-level fitting. To ensure we obtained accurate individual-level estimates of the ED50, we required that the 95% CI for the individual-level fit of the parameter x0 span less than one log10-unit (i.e., a factor of 10) from minimum to maximum. ED50 distributions were checked for potential bimodality by using kurtosis values.

Contrast analyses were performed to test for possible order effects of experimental light conditions. All pair-wise intergroup comparisons were tested for ED50, DLM0, and phase angle between DLM0 and habitual bedtime. In addition, we tested for Spearman correlations between individual-level ED50 values and (i) total baseline melatonin production (using average production after DLM0), (ii) DLM0 time, and (iii) phase angle between DLM0 and habitual bedtime.

Timing of apparent melatonin onset under experimental light conditions was computed for a 4-pGML threshold, with the same method used for computing DLM0. For each experimental light level, we computed the percentage of individuals who had achieved apparent melatonin onset as a function of time after the control-condition DLM0, in 1-h steps from 0 up to 4 h after baseline DLM0. For each individual, we estimated the photopic illuminance that would correspond to apparent melatonin onset occurring 1 h after DLM0. This was obtained by logarithmically transforming illuminance values, performing linear interpolation, and finding the minimum photopic illuminance at which a 1-h change would be predicted.