Integration of brief light flashes varying in intensity and duration by the human circadian system

Daniel S. Joyce[1, 2, 3, *] [0000-0002-6407-4653], Manuel Spitschan[1, 4, 5, 6, *] [0000-0002-8572-9268], & Jamie M. Zeitzer[1,2,] [0000-0001-6174-5282]

[1] *Department of Psychiatry and Behavioral Sciences, Stanford University*, Stanford, USA

[2] *Mental Illness Research Education and Clinical Center, VA Palo Alto Health Care System*, Palo Alto, USA

[3] *Department of Psychology, University of Nevada, Reno*, Reno, USA

[4] *Department of Experimental Psychology, University of Oxford*, United Kingdom

[5] *Centre for Chronobiology, Psychiatric Hospital of the University of Basel*, Switzerland

[6] *Transfaculty Research Platform Molecular and Cognitive Neurosciences, University of Basel,* Switzerland

[*] These authors contributed equally (shared first author).

Correspondence addresses: Daniel Joyce: djoyce@unr.edu, Manuel Spitschan: manuel.spitschan@psy.ox.ac.uk, Jamie Zeitzer: jzeitzer@stanford.edu

Abstract

The human circadian system is exquisitely sensitive to light, through a pathway connecting the melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) to the hypothalamic suprachiasmatic nuclei (SCN). ipRGCs are characterised by a delayed off-time following cessation of light exposure; we exploited this unusual physiologic property and examined how a sequence of flashes of bright light differing in intensity or duration presented in the biological night could delay the human circadian clock *in vivo* in healthy young participants (n=54). To understand the mechanism underlying circadian photoreception, we probed temporal integration by manipulating flash intensity and duration independently. In a 34-hour in-laboratory between-subjects design, we examined variable-intensity (3, 30, 95, 300, 950, 3000, or 9500 photopic lux; n=28 participants) flashes at fixed duration (2 ms), and variable-duration (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec) flashes at fixed intensity (2000 photopic lux; n=31 participants). We measured the phase shift of dim-light melatonin onset on the subsequent evening, and acute melatonin suppression and alertness during the flash sequence. In the variable-intensity study, we find a clear sigmoidal dose-response relationship for flash intensity and the induced circadian phase shift. In the variable-duration study, we find no parametric relationship between flash duration and induced circadian phase shift, indicating a relative insensitivity of the circadian system to flashes varying in duration. As the intermittent periods of darkness in our stimulation paradigm supports the recovery of extrinsic rod-cone signalling into the ipRGCs, our results strongly suggest rod-cone contributions into circadian photoreception.

Author Contributions

Conceptualization: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Formal Analysis: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Funding Acquisition: Jamie M. Zeitzer.

Investigation: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Methodology: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Project Administration: Jamie M. Zeitzer.

Resources: Jamie M. Zeitzer.

Software: Daniel S. Joyce and Manuel Spitschan.

Supervision: Jamie M. Zeitzer.

Visualization: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Writing – Original Draft Preparation: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Writing – Review & Editing: Daniel S. Joyce, Manuel Spitschan and Jamie M. Zeitzer.

Acknowledgements

This work was supported by United States Department of Defense (NNo to J.M.Z.). M.S. is supported by a Sir Henry Wellcome Fellowship (Wellcome Trust 204686/Z/16/Z) and a Junior Research Fellowship from Linacre College, University of Oxford. The authors would like to thank Yvonne Quevedo, Cheng-Ann Wang and Steven Lai for recruiting participants and conducting the experimental sessions, Chun-Ping (Phoebe) Liao for melatonin analysis, and the Stanford Product Realization Lab (PRL) for assistance with developing the light delivery system.

Introduction

The human circadian system is exquisitely sensitive to light. Light exposure in the evening and night can acutely suppress the production of melatonin [1-6], shift the phase of the circadian clock [5, 7-11], and modulate alertness and vigilance [12-14]. This effect is mediated by the retinal photoreceptors, with a major role played by a subset (<3%) of the retinal ganglion cells that express the short-wavelength-sensitive photopigment melanopsin, rendering them intrinsically photosensitive (ipRGCs = intrinsically photosensitive retinal ganglion cells) [15]. ipRGCs also receive cone and rod input [16], which contribute to a complex signal driving the circadian system. The exact effect of a given light on the circadian system depends on its intensity, spectral distribution, and duration [17-19]. While experimental durations of light exposure are typically on the order of hours, it has been shown that sequences of 2-millisecond flashes of bright light (~1,700 lux) can induce phase shifts in humans that are substantially larger than continuous light of the same illuminance [20].

Here, we systematically investigated the temporal integration properties of the human circadian system in a 34-hour in-laboratory between-subjects design. During the biological night, we exposed healthy observers (n=28) to a sequence of short-duration white light flashes that varied in flash intensity over 4.5 orders of magnitude (3, 30, 95, 300, 950, 3000, or 9500 photopic lux) at fixed duration (2 ms) and measured the consequent impacts on circadian phase, melatonin suppression, and alertness. Additionally, we examined how short of a flash the human circadian system could respond to by examining sequences of short-duration light flashes spanning 6 orders of magnitude (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec) at fixed intensity (2000 lux). Our results provide a mechanistic insight into the question how the human circadian system integrates environmental information of ambient illumination.

Results

Circadian phase shifts to flashed lights are intensity-dependent and robust. Light flashes shift the circadian clock in an intensity-dependent manner such that increasing illuminance increases the magnitude of circadian phase shift. In fitting a four-parameter log model to the data [21, 22], we found a 41 minute phase delay as a maximum response to light (Fig. **1*a***). The log-linear rise in response (i.e., the light intensities at which there is the greatest rate of change in phase shift per change in illuminance) occurred between an EC_{10} of 8.13 lux and an EC_{90} of 28.5 lux. Half-maximal response EC_{50}, the sensitivity of the system, occurred at 15.2 lux. In

contrast, previous work examining 6.5 hours of continuous light indicated an EC_{50} sensitivity of 119 lux with an EC_{10} and EC_{90} of 25 lux and 559 lux respectively [5]. Our results clearly demonstrate that flashes induce a circadian phase shift compared to a control condition without an active stimulus (examined in [23] using a parallel paradigm).

Circadian phase shifts to flashed lights are duration-independent and non-robust. With flashes of varying intensity showing a clear dose-dependent relationship with circadian phase shift, we examined the effect of a regime of 240 moderately bright flashes (~2,000 lux) of broad spectrum white light of varying duration (individual flash lengths of 10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 sec, 10 sec; 15 second duration onset-to-onset). On average, we find phase delays in circadian timing on the order of 18±36 minutes ($t(26) = 2.5434$, $p = 0.0173$). Surprisingly, the effect is independent of the duration of flashes (Figure **1*b***; estimated slope in simple linear intercept-slope model = 0.0025±0.061, p=0.97) over the entire range of durations, spanning six orders of magnitude (1:1,000,000).

Acute effects of flashed light are not dose-dependent. Salivary melatonin was not suppressed by the flashed lights and its expression did not change in a dose-dependent manner for either flash intensity (14.8±20.4% increase over the hour; $t(26) = 2.46$, $p = 0.021$; linear slope = 1.81±4.35, $p = 0.68$) or flash duration (average 11.3%±21.5% increase over the hour; $t(26) = 2.72$, $p = 0.011$; linear slope = -0.33±2.17, p=0.88). Median reaction time on the auditory PVT did not change in response to flash intensity (15.7±62.9 ms decrease over the hour, $t(25) = -1.2689$, $p = 0.22$, linear slope = -0.33±11.5, $p = 0.98$) or flash duration (23.1±66.7 ms decrease over the hour, $t(26) = -1.80$, $p = 0.083$, linear slope = -2.20±6.73, $p = 0.75$). Similarly, auditory PVT lapses reduced over the hour but were invariant to flash intensity (1.42±3.44 decrease over the hour, $t(25) = -2.11$, $p = 0.045$, linear slope = 0.39±0.63, $p = 0.54$) or flash duration (1.82±4.20 decrease over the hour, $t(26) = -2.25$, $p = 0.033$, linear slope = –0.31±0.42, $p = 0.47$). Subjective sleepiness measured by the SSS decreased over the hour but was invariant to flash intensity (average 1.15±1.67 point decrease; $t(25) = -3.53$, $p = 0.0016$, linear slope 0.26±0.28, $p = 0.36$) or flash duration (average 0.74±1.6 point decrease; $t(26) = -2.47$, $p = 0.020$, linear slope -0.14±0.15, $p = 0.37$).

A sweet spot for flashed lights? We examined the extent to which duration and intensity can be traded off in the circadian response. We collapsed our two data sets by calculating a common intensity-duration factor by multiplying illuminance by duration as well as the number of flashes (Fig. 2). We find that the totality of our data set reveals a nonlinear property of the human circadian system: With flashed stimuli, an optimal circadian response is found at

intermediate values for this intensity-duration metric, with smaller responses at higher (i.e., long duration/high intensity flashes) and lower (short duration/high intensity or long duration/low intensity flashes) values.

Discussion

As was implied in early studies of circadian photoreception [24], in response to brief flashes of light, the human circadian phase shifting system does not act as a photon counter. As with long duration (6.5 hour) continuous light exposure presented during the early biological night [21], our data demonstrate a sigmoidal dose-response relationship between flash intensity and magnitude of the phase shift. Circadian phase shifting is even more sensitive to light intensity in flashes than it is to continuous light, with a half-saturation constant nearly a full log unit lower (15 lux vs. 119 lux). Changes in the duration of the flash itself, however, seem to have little if any impact on strength of the light flash stimuli, as we observe invariant responses to a six-order of magnitude difference of flash durations. Our data are consistent with light flashes being mediated through mechanisms that differ from those mediating responses to continuous light.

Much of the impact of continuous light on circadian function is thought to be mediated through the intrinsic (melanopsin) rather than extrinsic (rod/cone) photoreceptive circuits, especially for monochromatic light [25]. Our data, however, suggest a possible mechanism for temporal integration is the relative increase in the outer retinal rod and/or cone contributions to circadian photoreception vision via the extrinsic ipRGC pathway. In rodents, outer retinal inputs to the SCN are both measurable [26] and functionally significant in driving photoentrainment, as melanopsin-knockout mice can stably entrain to an LD cycle but exhibit attenuated circadian phase shifting to a single pulse of monochromatic blue light [27]. An L+M-ON, S-OFF spatially coextensive spectral opponency has been identified for the extrinsic pathway of the M1 subtype in primates [28], but cone signalling rapidly adapts under continuous light paradigms that greatly reduces its ability to signal for NIF functions [29, 30]. For the circadian system at least, rod contributions may be preserved even at photopic irradiances and continue to drive photoentrainment [30, 31].

With the flashed light paradigm, the brief flashes in combination with the relatively long 15-second interstimulus intervals might allow rods to at least partially regenerate in the darkness [32, 33]. Because rods form the large majority of photoreceptors in the human retina, this contribution would not be insignificant. Future studies of *in vivo* ipRGC circuit

electrophysiology, coupled with human studies using monochromatic light or photoreceptor-selective stimulation paradigms [34, 35] (rather than polychromatic white light as used in this study) could clarify photoreceptor contributions to this process.

We note that the variability of individual responses to flashed light is higher than that of continuous light, but commensurate with other studies of flashed lights in humans [23, 36] and with the recently identified large individual differences in circadian sensitivities to evening light exposure [37]. In comparison to continuous light paradigms, flashed light paradigms may be more influenced by probabilistic photon catch over the short stimulus windows. The total stimulus duration over 1 hour is reduced 7,500-fold compared to an hour of continuous light or 48,750-fold compared to 6.5 hours of continuous light.

Flashed light confers advantages over continuous light when considering its acute effects on circadian physiology and behaviour. During the application of intermittent light, although subjective sleepiness (SSS) decreased by 1-point, objective RT did not, and salivary melatonin concentrations continued to rise. Importantly, there was no dose-response relationship with light intensity for any of these measures even though continuous light during the biological night results in dose-responses relationships between light intensity and subjective sleepiness, EEG theta spectral power density [38], and melatonin suppression [21]. The lack of a dose-response relationship, and that light stimuli only altered subjective sleepiness, together suggest that these findings are underpinned by psychological factors (i.e., being awakened at night to observe flashed light) rather than psychophysical factors (differences in sensations of the light intensities). The distinct subtypes of ipRGCs may also contribute to this observation, where differences in retinal connectivity, temporal, spatial and intensity signalling, coupled with both distinct and overlapping brain targets [39-45], may lead to divergent light sensitivities depending on the physiologic pathway(s) assayed through the outcome measure(s) selected.

This study is the first to define the intensity sensitivity of the circadian system to sequenced flashes of light presented during the biological night. This paradigm leverages evolutionarily unnatural stimuli to drive clinically meaningful shifts in circadian rhythms without substantial detriments to acute measures of sleep behaviour and circadian physiology, in contrast to continuous light that often affects such performance markers in undesirable ways. The flashed light paradigm is therefore a powerful method to drive clinically useful shifts in circadian rhythms, and, further, is orders of magnitude more efficient than continuous light paradigms in terms of time, energy and outcome, which is critical in the development of wearable technology

that could be developed as a countermeasure to circadian desynchrony in a variety of environments.

The ability of humans to consciously perceive light spans a very wide range of light intensities, from the sensitivity to single photons by the retinal rods [e.g. 46] to encoding fine spatial detail, colour and motion during daytime light levels. At detection threshold, the intensity and duration of a flash can be traded off against one another below a certain flash critical duration, leading to the same conscious perceptual performance if the product between the intensity and the duration is the same. For conscious perception, this temporal integration does not hold for flash durations over ~100 ms [47]. For shifting the circadian clock, however, it appears as though the mechanisms integrating light information are different and are likely non-linear, as shown in the results here.

As we continue to develop the technical abilities to craft light in terms of colour and intensity, the manipulation of temporal properties of light may become the new frontier to improve health and well-being. Our study shows how in principle, ultra-brief light flashes at the microsecond scale can be used to elicit phase shifts no different in magnitude than a regime of flashes delivering 100,000 times as many photons. Exploiting this surprising duration-invariance of human circadian photoreception could be used to address circadian desynchrony emerging in modern life, such as jet lag or shift work, in an energy-efficient way. Our results indicate that longer is not always better for the circadian system.

Materials and Methods

Pre-registration and deviations from pre-registered protocol. The study protocol registered at ClinicalTrials.gov (NCT01119365; "Bright Light as a Countermeasure for Circadian Desynchrony"). The variable-duration study was pre-registered on the Open Science Framework (https://osf.io/5sv53/). Notably, we deviated from the pre-registered study protocol by including an additional (10 sec) exposure duration. In both the variable-intensity and the variable-duration studies, polysomnography (PSG) data were collected but not analysed.

Ethical approval. The protocol was reviewed and approved by the Stanford University Institutional Review Board, conforming to the Declaration of Helsinki. Prior to any procedures, subjects signed informed consent forms.

Sample characteristics. A total of 59 healthy, young (18-35 years) participants of normal weight with no somatic diseases, sleep disorders (Pittsburgh Sleep Quality Index, PSQI [48] ≤ 5), moderate chronotype (reduced Morningness-Eveningness Questionnaire, MEQ [49] ≥ 11 and ≤ 27), no history of substance abuse (Alcohol Use Disorders Identification Test, AUDIT [50] ≤ 7), no depressive symptoms (Center for Epidemiologic Studies Depression scale, CES-D [51] ≤ 17), no use of hormonal contraceptives (females only), and normal colour vision (assessed with Ishihara Plates [52]) completed the studies. Females attended the lab within four days after the onset of menses.

Variable-intensity study. 28 participants (14 female, 14 male) completed the study. We excluded one participant from further analyses due to mistiming of the light stimulus relative to their circadian phase, yielding a total sample of 27 subjects (n=27, mean±1SD age: 27±5.16) years. The break-down of intensity assignment was: 3 lux, 4 participants; 30 lux, 4 participants; 95 lux, 4 participants; 300 lux, 3 participants; 950 lux, 4 participants; 3000 lux, 4 participants; 9500 lux, 4 participants.

Variable-duration study. 31 participants (13 female, 18 male) completed the study. We excluded two participants (1 female, 1 male) because of contaminated melatonin assays, one participant due to mistiming of light (female), and one participant because of accidental light exposure in the morning (female), yielding a total sample of 27 subjects (n=27, mean±1SD age: 25.7±3.94 years). The break-down of duration assignment was: 10 μs, 3 participants; 100 μs, 4 participants; 1 ms, 4 participants; 10 ms, 5 participants; 100 ms, 3 participants; 1 s, 4 participants; 10 s, 4 participants.

Design. Participants were exposed to a sequence of 240 light flashes of varying, logarithmically spaced intensity at fixed duration (2 ms flashes; 3, 30, 95, 300, 950, 3000, or

9500 photopic lux), or varying duration at fixed intensity (10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 s, 10 s, 2000 lux) spaced 15 seconds apart (from onset to onset). Acute effects on melatonin suppression, objective alertness, subjective sleepiness, and electrophysiological correlates of arousal (polysomnography, PSG) were measured immediately before and at the end of the light exposure (LE). Effects of LE on circadian phase was measured as the change in melatonin onset determined on a constant posture protocol (CP1) prior to light exposure and a constant posture protocol the following day (CP2).

Protocol. Participants took take part in a 16-day study protocol:

Days 1-14: Participants were instructed to maintain a regular sleep and wake time schedule at home (±30 minute window of bed time and wake time). Sleep-wake patterns were monitored using an actigraph (Actiwatch2, Philips, Bend OR) and a self-reported sleep diary [53]. From these data, the midpoint of sleep (MSP) was estimated and used as the midpoint of the in-laboratory sleep opportunity.

Day 15: The participant entered the laboratory during the late afternoon of Day 15. During the evening, the participant underwent the first constant posture procedure (CP1, 8 hour duration, beginning 8 hours before habitual bedtime). During this procedure, the participant was given isocaloric meals (Ensure, Abbott Laboratories, Chicago IL) every 60 minutes, yielding a total caloric intake matched to what they would have received during dinner (calculated using the Mifflin-St. Jeor formula; [54]). During CP1, objective alertness (auditory version of the Psychomotor Vigilance Task, PVT; [55, 56]) and subjective sleepiness (Stanford Sleepiness Scale, SSS; [57]) were measured every 60 minutes. Saliva was collected every 30 minutes in untreated polypropylene tubes. Four hours before MSP (typical bedtime), participants were given the opportunity to sleep in darkness. After 1 hour and 45 minutes of sleep time in darkness (2 hours and 15 minutes before MSP), participants were awakened in the dark. Saliva was collected and auditory PVT and SSS were administered in the dark. Starting two hours before MSP, participants were exposed to a sequence of 240 full-field flashes through a custom-made mask for light delivery. At 20, 40 and 60 minutes into the light exposure, saliva was collected. During the last 10 minutes of light exposure, the auditory PVT was administered, followed by an SSS. The mask was then removed from the participant and the participant continued to sleep in darkness.

Day 16: The participant was awakened at their habitual wake time (4 hours after MSP) into a dimly light room (<10 lux) and received breakfast and lunch at usual times. During the evening, the participant had a second constant posture procedure (CP2, 10 hours in total, beginning 8

hours before habitual bedtime), during which the participant was given isocaloric meals every 60 minutes, yielding a total caloric intake matched to what they would have received during dinner (same number as on Day 15, but spread over 10 hours instead of 8). During CP2, objective alertness (auditory PVT) and subjective sleepiness (SSS) were measured every 60 minutes and saliva was collected every 30 minutes.

Stimulus delivery. Binocular full-field flashes of differing durations (2 ms in variable-intensity study; 10 μs, 100 μs, 1 ms, 10 ms, 100 ms, 1 s, 10 s in variable-duration study) were delivered to the observer. A custom-light mask was constructed using modified welding goggles (Jackson WS-80 Shade 5.0 Cutting Goggles; Kimberly-Clark Professional, Mississauga, ON, Canada) containing an acrylic panel with three horizontally arranged LED strips (12 SMD LEDs each, Lumileds L235-4080AHLCBAAC0). The light from the LEDs was diffused using a piece of diffusing acrylic (TAP Plastics, Mountain View, CA). For additional diffusion, the participant wore ping pong ball halves cut out to match the shape of the eye's orbit. The LEDs were pulsed using electronics developed in-house based on the Arduino Uno R3 microcontroller. In the variable-intensity study, we used we verified photopic illuminances at 3, 30, 95, 300, 950, 3000, or 9500 photopic lux as confirmed by a calibrated photometer (International Light Technologies ILT900, Peabody, MA, USA).

We verified the timing of our apparatus for the nominal flash durations 10 μs, 100 μs, 1 ms, 10 ms, 100 ms, and 1 s using an integrated photodiode and transimpedance amplifier (OPT101, Texas Instruments) connected to a digital oscilloscope (Tektronix TDS 2024C). We measured the logic-level control pulse sent from the microcontroller as well as the light output (Fig. S1). We averaged over 128 (10 μs), 128 (100 μs), 128 (1 ms), 64 (10 ms), 16 (100 ms), and 16 (1 s) pulses. The maximum amplitude of the pulse is approximately constant across all nominal pulse durations, indicating that there is no shift in light intensity due to duration. Integrating the light output, the logarithm of the integrated light output over the pulse duration is linear with the logarithm of the nominal pulse duration. Spectral output was measured using a PR-670 spectroradiometer (Photo Research, Syracuse NY) (yielding calibrated radiance) for all stimulus durations, indicating stationarity of the spectrum across stimulus durations and across the entire stimulus protocol (240 flashes, 1h). The results of these validation measurements are shown in Figure S2.

Spectral and α-opic properties of light. The spectrum of the light measured in the corneal plane corresponded to a warmish white light (CIE 1931 xy chromaticity 0.4092, 0.3969; correlated colour temperature [CCT] 3466K). The spectrum is visualised in Figure S1, and

310 tabulated as a relative spectrum in Supplementary Table S1. Metrics related to the recent CIE
311 S026/E:2018 standard are given in Table 1 [58]. The spectral invariance with stimulus duration
312 is shown in Figure S2.

313 ***Melatonin assay.*** Saliva (at least 1 mL) was collected using polypropylene tubes (Fisher
314 Scientific, Hampton NH). Samples were centrifuged after collection, frozen at –20°C, then
315 stored at –80°C within 24 hours. Salivary melatonin concentration was assayed according to
316 the manufacturer's instructions (Salivary melatonin ELISA #3402, Salimetrics, Carlsbad CA;
317 assay range: 0.78-50 pg/mL, sensitivity = 1.37 pg/mL). For a given participant, all samples
318 were assayed on the same plate.

319 ***Objective alertness: auditory PVT.*** We used a modified auditory psychomotor vigilance test
320 (PVT; [55, 56]) to measure objective alertness using a serial collection of simple reaction times
321 to auditory stimuli generated by a piezo buzzer. The stimuli were spaced apart in time at
322 random inter-stimulus intervals (ISIs) between 2 and 6 seconds (discrete steps: 2, 3, 4, 5, 6
323 second ISIs). Upon button press, the tone stopped and the next trial began with a random ISI.
324 Approximately 100 of these stimuli were presented, with the order of ISIs randomized at the
325 beginning of the experiment. This assessment took 10 minutes. The auditory PVT was
326 implemented using custom-made Arduino hardware and software. To measure response
327 latencies, we modified sample code from a report validating using the Arduino platform to
328 measure reaction times [59]. There is a 30-second time out which is considered a lapse trial. If
329 there is a response during the ISI period, this was counted as an error of commission and the
330 counter was reset, starting a new trial period. The random seed for the ISIs is initialized by
331 reading analogue voltage noise from an unconnected pin in the Arduino.

332 ***Subjective alertness: SSS.*** Participants completed the Stanford Sleepiness Scale (SSS, [57]).
333 The SSS is a single question assessment of current sleepiness that uses a 7-point Likert-like
334 scale. Scale values range from 1 to 7, with higher values indicating greater subjective
335 sleepiness.

336 ***Determination of phase shift.*** Phase shifts were determined by examining the acute change in
337 the timing of salivary DLMO. This onset was determined by calculating the time at which the
338 melatonin concentrations rose above a variable threshold (twice the average of the first three
339 daytime samples [60]). In cases in which this variable threshold was ambiguous (n=1 variable-
340 intensity; n=4 in variable-duration study; 5/59 participants in total = 8.4%), we used a the
341 hockey-stick curve-fitting method [61]. Determination of ambiguity was made blind to the

lighting parameters to which the participant was exposed. Phase shift was calculated as the DLMO on CP1 – DLMO on CP2, such that negative changes indicate a delay in timing.

Statistical analysis. We subjected our data to two sets of statistical analysis. For each measure, we estimated if there was a non-zero slope using a simple linear model with an intercept and slope term, with $\log_{10}$ flash duration as the predictor. A non-zero slope would indicate a monotonic relationship between flash duration and the measure. Additionally, we used single-sample *t*-tests on change scores to determine if there is any effect at all, irrespective of flash duration. The measures of interest were: phase shift (Fig. 2A), difference in melatonin pre- and post-LE (Fig. 2B), the difference in number of lapses in the auditory PVT pre- and post-LE (Fig. S1A), the difference in median reaction time pre- and post-LE (Fig. S1A), and the difference in sleepiness as assessed by SSS (Fig. S1B). Data are presented as mean±SD.

Figures

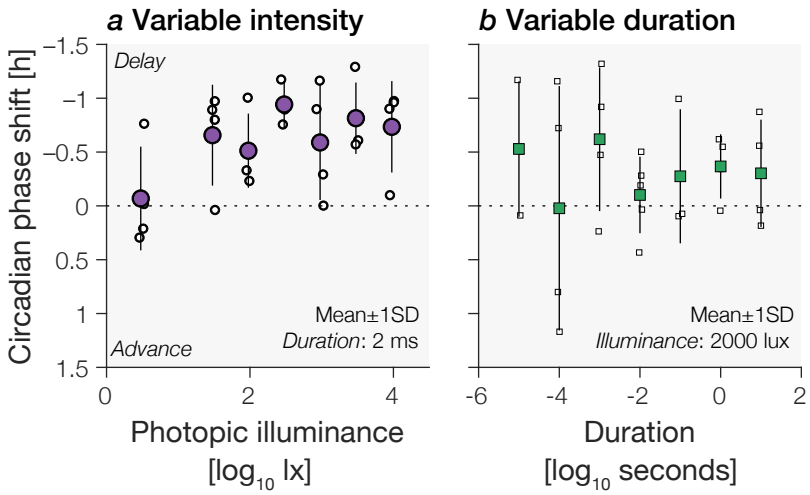

Figure 1. ***Flashes of light shift circadian phase in a dose-dependent manner. a*** Dose-response curve for circadian phase shifts across 3.5 orders of magnitude of photopic illuminance (3-9,500 lx; 2 ms flashes) measured in an in-laboratory between-subjects design (n=27). ***b*** Dose-response curve for circadian phase shifts across 6 orders of magnitude of flash duration (10 μs-10 s; 2000 lx flashes) measured in an in-laboratory between-subjects design (n=27).

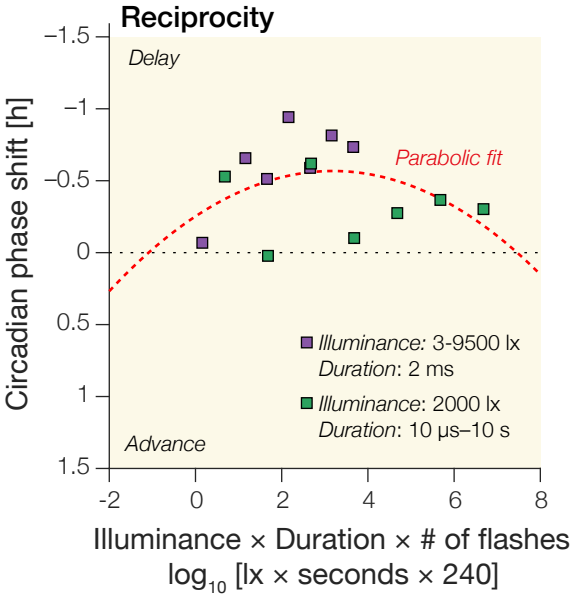

Figure 2. ***Reciprocity relationships.*** Circadian phase shifts induced by temporally flashing lights appear to follow an inverted U-curve as a function of the illuminance × duration product.

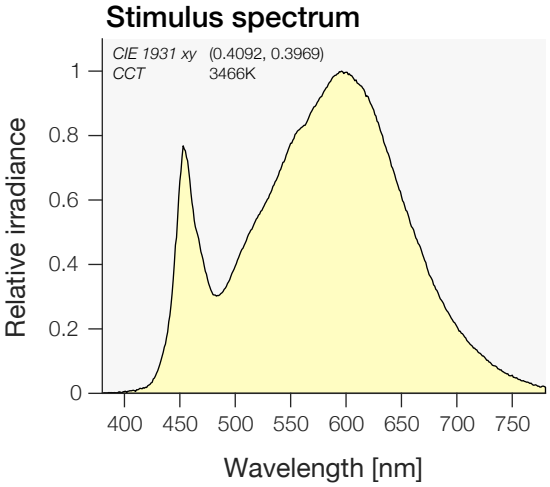

Figure S1. ***Stimulus spectrum (normalised to maximum irradiance).*** CCT was estimated using Psychtoolbox's SPDToCCT function [62].

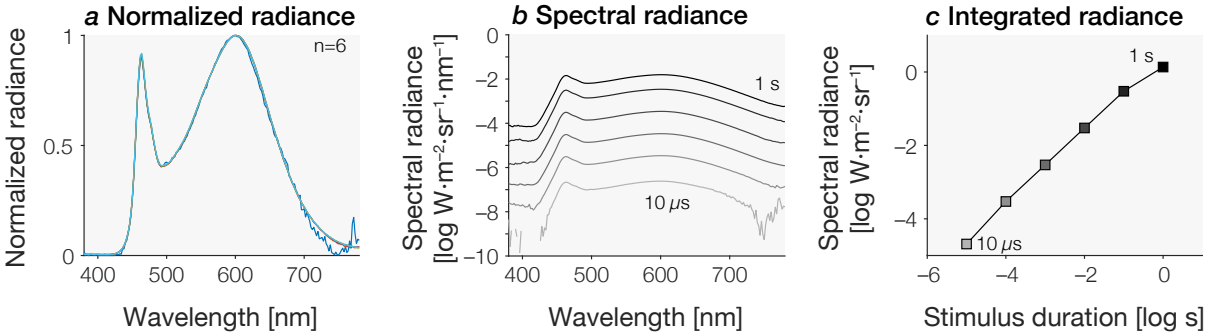

Figure S2. ***Spectral invariance across flash durations. a*** Time-averaged irradiance spectra measured across six flash durations (10 μs-10 s, 2000 lx). The spectra are perfectly overlaying apart from long-wavelength noise for the lowest duration due to measurement noise at these low intensities. ***b*** Time-averaged irradiance spectra shown on a logarithmic scale. The noise in the short- and long-wavelength ends of the spectrum (>750 nm and <450 nm) appear amplified here but are inconsequential and represent amplified noise. The broken spectrum is due to the spectrometer reporting 0. ***c*** Time-averaged and spectrally integrated radiance, demonstrating linearity in spectrum across flash durations.

Tables

	S-cone-opic	**M-cone-opic**	**L-cone-opic**	**Rhodopic**	**Melanopic**
α-opic irradiance, [mW·m^{-2}]	0.35	1.2	1.6	0.90	**0.72**
α-opic efficacy of luminous radiation, [mW·lm^{-2}]	0.35	1.2	1.6	0.90	**0.72**
α-opic equivalent daylight (D65) illuminance [lx]	0.43	0.85	1.0	0.62	**0.55**

Table 1: α-opic stimulus properties at unit photopic illuminance (1 lux) calculated using the free CIE S 026 α-opic Toolbox (v1.049, version dated 26 March 2020) implementing the CIE S 026/E:2018 standard [58]. To derive the α-opic irradiance and the α-opic equivalent daylight (D65) illuminance at other photopic illuminances, multiply the values by the photopic illuminance value. The α-opic efficacy of luminous radiation is a scale-invariant ratio that only depends on the relative spectrum.

References

1. Lewy, A.J., et al., *Light suppresses melatonin secretion in humans.* Science, 1980. **210**(4475): p. 1267-9.
2. Thapan, K., J. Arendt, and D.J. Skene, *An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans.* J Physiol, 2001. **535**(Pt 1): p. 261-7.
3. Prayag, A.S., R.P. Najjar, and C. Gronfier, *Melatonin suppression is exquisitely sensitive to light and primarily driven by melanopsin in humans.* J Pineal Res, 2019. **66**(4): p. e12562.
4. Brainard, G.C., et al., *Dose-response relationship between light irradiance and the suppression of plasma melatonin in human volunteers.* Brain Res, 1988. **454**(1-2): p. 212-8.
5. Zeitzer, J.M., et al., *Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression.* J Physiol, 2000. **526 Pt 3**: p. 695-702.
6. Brainard, G.C., et al., *Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor.* J Neurosci, 2001. **21**(16): p. 6405-12.
7. Czeisler, C.A., et al., *Bright light induction of strong (type 0) resetting of the human circadian pacemaker.* Science, 1989. **244**(4910): p. 1328-33.
8. Khalsa, S.B.S., et al., *The timing of the human circadian clock is accurately represented by the core body temperature rhythm following phase shifts to a three-cycle light stimulus near the critical zone.* J Biol Rhythms, 2000. **15**(6): p. 524-30.
9. Klerman, E.B., et al., *Circadian phase resetting in older people by ocular bright light exposure.* J Investig Med, 2001. **49**(1): p. 30-40.
10. Minors, D.S., J.M. Waterhouse, and A. Wirz-Justice, *A human phase-response curve to light.* Neurosci Lett, 1991. **133**(1): p. 36-40.
11. Shanahan, T.L., J.M. Zeitzer, and C.A. Czeisler, *Resetting the melatonin rhythm with light in humans.* J Biol Rhythms, 1997. **12**(6): p. 556-67.
12. Lockley, S.W., et al., *Short-wavelength sensitivity for the direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans.* Sleep, 2006. **29**(2): p. 161-8.
13. Cajochen, C., et al., *High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light.* J Clin Endocrinol Metab, 2005. **90**(3): p. 1311-6.
14. Cajochen, C., et al., *Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness.* Behav Brain Res, 2000. **115**(1): p. 75-83.
15. Provencio, I., et al., *A novel human opsin in the inner retina.* J Neurosci, 2000. **20**(2): p. 600-5.

423 16. Dacey, D.M., et al., *Melanopsin-expressing ganglion cells in primate retina signal colour and*
424 *irradiance and project to the LGN.* Nature, 2005. **433**(7027): p. 749-54.
425 17. Rahman, S.A., et al., *Circadian phase resetting by a single short-duration light exposure.* JCI
426 Insight, 2017. **2**(7): p. e89494.
427 18. Chang, A.M., et al., *Human responses to bright light of different durations.* J Physiol, 2012.
428 **590**(13): p. 3103-12.
429 19. Gronfier, C., et al., *Efficacy of a single sequence of intermittent bright light pulses for*
430 *delaying circadian phase in humans.* Am J Physiol Endocrinol Metab, 2004. **287**(1): p. E174-
431 81.
432 20. Najjar, R.P. and J.M. Zeitzer, *Temporal integration of light flashes by the human circadian*
433 *system.* J Clin Invest, 2016. **126**(3): p. 938-947.
434 21. Zeitzer, J.M., et al., *Sensitivity of the human circadian pacemaker to nocturnal light:*
435 *Melatonin phase resetting and suppression.* The Journal of Physiology, 2000. **526**(3): p. 695-
436 702.
437 22. Nelson, D.E. and J.S. Takahashi, *Sensitivity and integration in a visual pathway for circadian*
438 *entrainment in the hamster (Mesocricetus auratus).* The Journal of Physiology, 1991. **439**(1):
439 p. 115-145.
440 23. Zeitzer, J.M., et al., *Response of the human circadian system to millisecond flashes of light.*
441 PloS ONE, 2011. **6**(7): p. e22078.
442 24. Nelson, D.E. and J.S. Takahashi, *Sensitivity and integration in a visual pathway for circadian*
443 *entrainment in the hamster (Mesocricetus auratus).* J Physiol, 1991. **439**: p. 115-45.
444 25. Brown, T.M., *Melanopic illuminance defines the magnitude of human circadian light*
445 *responses under a wide range of conditions.* J Pineal Res, 2020: p. e12655.
446 26. Berson, D.M., F.A. Dunn, and M. Takao, *Phototransduction by retinal ganglion cells that set*
447 *the circadian clock.* Science, 2002. **295**(5557): p. 1070-1073.
448 27. Panda, S., et al., *Melanopsin (Opn4) requirement for normal light-induced circadian phase*
449 *shifting.* Science, 2002. **298**(5601): p. 2213-2216.
450 28. Dacey, D.M., et al., *Melanopsin-expressing ganglion cells in primate retina signal colour and*
451 *irradiance and project to the LGN.* Nature, 2005. **433**: p. 749-754.
452 29. Gooley, J.J., et al., *Melanopsin and rod–cone photoreceptors play different roles in mediating*
453 *pupillary light responses during exposure to continuous light in humans.* The Journal of
454 Neuroscience, 2012. **32**(41): p. 14242-14253.
455 30. Lall, G.S., et al., *Distinct contributions of rod, cone, and melanopsin photoreceptors to*
456 *encoding irradiance.* Neuron, 2010. **66**(3): p. 417-428.
457 31. Altimus, C.M., et al., *Rod photoreceptors drive circadian photoentrainment across a wide*
458 *range of light intensities.* Nature Neuroscience, 2010. **13**(9): p. 1107-1112.

32. Rushton, W.A. and D.S. Powell, *The rhodopsin content and the visual threshold of human rods.* Vision Res, 1972. **12**(6): p. 1073-81.
33. Tikidji-Hamburyan, A., et al., *Retinal output changes qualitatively with every change in ambient illuminance.* Nat Neurosci, 2015. **18**(1): p. 66-74.
34. Spitschan, M. and T. Woelders, *The method of silent substitution for examining melanopsin contributions to pupil control.* Front Neurol, 2018. **9**: p. 941.
35. Spitschan, M., et al., *No evidence for an S cone contribution to acute neuroendocrine and alerting responses to light.* Curr Biol, 2019. **29**(24): p. R1297-R1298.
36. Najjar, R.P. and J.M. Zeitzer, *Temporal integration of light flashes by the human circadian system.* Journal of Clinical Investigation, 2016. **126**(3): p. 938-947.
37. Phillips, A.J.K., et al., *High sensitivity and interindividual variability in the response of the human circadian system to evening light.* Proceedings of the National Academy of Sciences, 2019. **116**(24): p. 12019-12024.
38. Cajochen, C., et al., *Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness.* Behavioural Brain Research, 2000. **115**(1): p. 75-83.
39. Schmidt, T.M., S.K. Chen, and S. Hattar, *Intrinsically photosensitive retinal ganglion cells: Many subtypes, diverse functions.* TRENDS in Neurosciences, 2011. **34**(11): p. 572-580.
40. Schmidt, T.M. and P. Kofuji, *Differential cone pathway influence on intrinsically photosensitive retinal ganglion cell subtypes.* The Journal of Neuroscience, 2010. **30**(48): p. 16262-16271.
41. Do, M.T.H. and K.-W. Yau, *Adaptation to steady light by intrinsically photosensitive retinal ganglion cells.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(18): p. 7470-7475.
42. Hannibal, J., et al., *Central projections of intrinsically photosensitive retinal ganglion cells in the macaque monkey.* Journal of Comparative Neurology, 2014. **522**(10): p. 2231-2248.
43. Milner, E.S. and M.T.H. Do, *A population representation of absolute light intensity in the mammalian retina.* Cell, 2017. **171**(4): p. 865-876.e16.
44. Zhao, X., et al., *Photoresponse diversity among the five types of intrinsically photosensitive retinal ganglion cells.* The Journal of Physiology, 2014. **592**(7): p. 1619-1636.
45. Liao, H.-W., et al., *Melanopsin-expressing ganglion cells on macaque and human retinas form two morphologically distinct populations.* Journal of Comparative Neurology, 2016. **524**: p. 2845-2872.
46. Hecht, S., S. Shlaer, and M.H. Pirenne, *Energy, Quanta, and Vision.* J Gen Physiol, 1942. **25**(6): p. 819-40.
47. Barlow, H.B., *Temporal and spatial summation in human vision at different background intensities.* J Physiol, 1958. **141**(2): p. 337-50.

496 48. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric*
497 *practice and research.* Psychiatry Res, 1989. **28**(2): p. 193-213.
498 49. Adan, A. and H. Almirall, *Horne & Östberg morningness-eveningness questionnaire: A*
499 *reduced scale.* Pers Individ Dif, 1991. **12**(3): p. 241-253.
500 50. Saunders, J.B., et al., *Development of the Alcohol Use Disorders Identification Test (AUDIT):*
501 *WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol*
502 *Consumption--II.* Addiction, 1993. **88**(6): p. 791-804.
503 51. Radloff, L.S., *The CES-D Scale.* Applied Psychological Measurement, 1977. **1**(3): p. 385-
504 401.
505 52. Ishihara, S., *Ishihara's Tests for Colour Deficiency*. 2007, Tokyo: Kanehara Trading Inc.
506 53. Carney, C.E., et al., *The consensus sleep diary: standardizing prospective sleep self-*
507 *monitoring.* Sleep, 2012. **35**(2): p. 287-302.
508 54. Mifflin, M.D., et al., *A new predictive equation for resting energy expenditure in healthy*
509 *individuals.* Am J Clin Nutr, 1990. **51**(2): p. 241-7.
510 55. Dinges, D.F. and J.W. Powell, *Microcomputer analyses of performance on a portable, simple*
511 *visual RT task during sustained operations.* Behav Res Meth Instrum Comput, 1985. **17**(6): p.
512 652-655.
513 56. Gabel, V., et al., *Auditory psychomotor vigilance testing in older and young adults: a revised*
514 *threshold setting procedure.* Sleep Breath, 2019. **23**(3): p. 1021-1025.
515 57. Hoddes, E., et al., *Quantification of sleepiness: a new approach.* Psychophysiology, 1973.
516 **10**(4): p. 431-6.
517 58. CIE, *CIE S 026/E:2018: CIE System for Metrology of Optical Radiation for ipRGC-*
518 *Influenced Responses to Light*. 2018, CIE Central Bureau: Vienna, Austria.
519 59. Schubert, T.W., A. D'Ausilio, and R. Canto, *Using Arduino microcontroller boards to*
520 *measure response latencies.* Behav Res Methods, 2013. **45**(4): p. 1332-46.
521 60. Voultsios, A., D.J. Kennaway, and D. Dawson, *Salivary melatonin as a circadian phase*
522 *marker: validation and comparison to plasma melatonin.* J Biol Rhythms, 1997. **12**(5): p.
523 457-66.
524 61. Danilenko, K.V., et al., *The hockey-stick method to estimate evening dim light melatonin*
525 *onset (DLMO) in humans.* Chronobiol Int, 2014. **31**(3): p. 349-55.
526 62. Brainard, D.H., *The Psychophysics Toolbox.* Spat Vis, 1997. **10**(4): p. 433-6.
527