YALE JOURNAL OF BIOLOGY AND MEDICINE 92 (2019), pp.337-348.

MINI-REVIEW

YALE JOURNAL OF BIOLOGY AND MEDICINE
YJBM
CELEBRATING 90 YEARS 1928–2018

Circadian Responses to Fragmented Light: Research Synopsis in Humans

Fabian Fernandez*

Departments of Psychology, Neurology, the BIO5 Institute, and Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ

Light is the chief signal used by the human circadian pacemaker to maintain precise biological timekeeping. Though it has been historically assumed that light resets the pacemaker's rhythm in a dose-dependent fashion, a number of studies report enhanced circadian photosensitivity to the initial moments of light exposure, such that there are quickly diminishing returns on phase-shifting the longer the light is shown. In the current review, we summarize findings from a family of experiments conducted over two decades in the research wing of the Brigham and Women's Hospital that examined the human pacemaker's responses to standardized changes in light patterns generated from an overhead fluorescent ballast. Across several hundred days of laboratory recording, the research group observed phase-shifts in the body temperature and melatonin rhythms that scaled with illuminance. However, as suspected, phase resetting was optimized when exposure occurred as a series of minute-long episodes separated by periods of intervening darkness. These observations set the stage for a more recent program of study at Stanford University that evaluated whether the human pacemaker was capable of integrating fragmented bursts of light in much the same way it perceived steady luminance. The results here suggest that ultra-short durations of light—lasting just 1-2 seconds in total—can elicit pacemaker responses rivaling those created by continuous hour-long stimulation if those few seconds of light are evenly distributed across the hour as discreet 2-millisecond pulses. We conclude our review with a brief discussion of these findings and their potential application in future phototherapy techniques.

INTRODUCTION

The circadian pacemaker is a biologically conserved timekeeping system that evolved to anticipate daily recurring changes in the environment produced by the solar light-dark cycle [1]. This anticipation grants organisms, from unicellular prokaryotic cyanobacteria to multicellular eukaryotes, the ability to optimize timing of activities critical for reproductive success and survival [2]. In mammals, the light and dark signals that serve to synchronize the central pacemaker are processed through the retinohypothalamic tract [3-5]. Photoreceptors in the retina provide the sole input into this pathway, which sends information about light transitions in the environment (*i.e.*, dawn and dusk) and photoperiod length directly to the central pacemaker neurons seated within the supra-

*To whom all correspondence should be addressed: Fabian Fernandez, PhD, Department of Psychology, 1501 N. Campbell Avenue Life Sciences North, Room 349, Tucson, Arizona, 85724; Email: FabianF@email.arizona.edu.

†Abbreviations: SCN, suprachiasmatic nucleus; CBT_{min}, core body temperature minimum; DLMO, dim-light melatonin onset; h, hour; LED, light-emitting diode; PRC, phase response curve.

Keywords: Circadian, Phase shifting, Phototherapy, Light treatment, Light fractionation, Millisecond flashes, Intermittent, Pulse

Author Contributions: FF planned the outline of the review, wrote the manuscript, and designed the figures.

Copyright © 2019

chiasmatic nucleus (SCN†) [6-8]. On a daily basis, light resets the SCN's imprecise circadian period to a period closely aligned with the 24-h rotation of the Earth, thereby entraining the circadian rhythms of the rest of the body to the light/dark schedule of the outside world [9]. The SCN uses both diffusible chemical signals and "hard-wired" (multisynaptic) neural circuitry to disseminate circadian cues to the periphery [10-12]. These cues organize peripheral oscillators operating in the body's organs so that major physiological processes—such as digestion and nutrient absorption—are coordinated with one another and optimized for times-of-day when, for example, food is most readily available [13,14].

THE PHASE RESPONSE CURVE TO LIGHT

The pacemaker's timekeeping adjustments to natural light are often modeled by a sinusoidal-like phase response curve (PRC) that has been documented in many species to date through the use of electric light sources (Figure 1a) [15]. Electric light administered in the first half of the evening invariably triggers a phase delay in one's subjective schedule of physiology and behavior, while later administration in the second half causes a phase advance (by convention, the magnitude of the delay and advance shifts mobilized by light are plotted with negative and positive numbers, respectively; Figure 1a) [16,17]. The shape of the PRC, which can be adapted to different seasonal photoperiods, likely reflects an entrainment logic designed to maintain the temporal niche of an animal [18]. If light in the early evening is perceived as an extension of the sunset—and that seen in the latter half the leading edge of a sunrise—then such phase regulation would maximize the contact that diurnal animals have with the sun and minimize the exposure of nocturnal animals. The amplitude characteristics of the PRC, though different in its particulars from one species to the next, bear out this suggestion.

The most sensitive parts of the delay and advance zones can be found several hours after dusk (Figure 1b) and several hours before dawn (Figure 1c), respectively. If electric light presented in these areas affects the circadian system the same as sunlight, then this would alter the pacemaker's interpretation of the solar cycle, forcing it to manufacture large 2-4-h phase jumps in order to realign the timing of its internally created day and night to the newly perceived twilight zones. This is, in fact, what happens after light stimulation in commonly used animal models such as *Drosophila*, rodents, as well as humans [16,19-23]. When electric light exposure occurs within the less sensitive parts of the PRC in the 1-2 h of the night bookending dusk (lights-off) or dawn (lights-on), smaller phase shifts commensurate in magnitude with the difference in timing between the photic stimulation and the offsets/onsets of the light schedule are also routinely observed (Figure 1b-c). The picture that emerges with the PRC is a simple one: with the addition of photoreceptor input, the central pacemaker has become an exquisitely sensitive sunrise and sunset detector. To withstand natural selection, nearly every surface dwelling animal on the planet was obliged to use the same exact data stream of twilight information to measure lapses in time, whether their species started with the necessary nervous system hardware (or clock molecules) to do so or not. Because of this, circadian pacemakers are considered an exemplar of convergent evolution [24].

ATTEMPTS TO EXPLAIN CIRCADIAN RESPONSES TO LIGHT THROUGH RECIPROCITY: ANIMAL STUDIES

Despite the progress that has been made in elucidating the nature of the molecular machinery responsible for circadian oscillations in cells [25-27], relatively little is known about how the pacemaker, as a "systems unit," samples from the naturally occurring light and darkness it receives, and then factors in differences in irradiance, spectral quality, and probability of exposure to deduce geophysical time. One strategy that many investigators have used to begin to deconstruct this logic is to assess what parameters guide the pacemaker's short-term reaction to nighttime photic stimulation with incandescent or cool fluorescent lamps [28]. Construction of electric light PRCs across a variety of animal models has continued to reaffirm that the single most important variable driving the magnitude and direction of a phase shift is the precise timing of light administration [15,29]. However, within the same timeframe of the subjective night, other photic variables have also emerged that *further* influence phase-shifting. These include the spectral weighting of a light pulse and its brightness or length [30,31], along with the light level or duration of lighting under which a person or animal is housed (*i.e.*, dim light histories in either case amplify phase-shifting responses to sub-saturating light stimuli) [32,33]. Based on knowledge regarding the biophysical properties of ocular photoreceptors, recent studies have expanded the parameter space in sophisticated ways by showing for instance that different sequences of monochromatic light designed to activate—and then re-sensitize—pacemaker targeting photoreceptors can augment phase resetting by maximizing information flow to the pacemaker [34,35].

Recent observations notwithstanding, the historical dogma that has organized much of the study on the pacemaker's relationship with light and darkness is summarized by the *reciprocity hypothesis*. It postulates that any shift made in response to light at a fixed point in the subjective night results solely from the number of photons

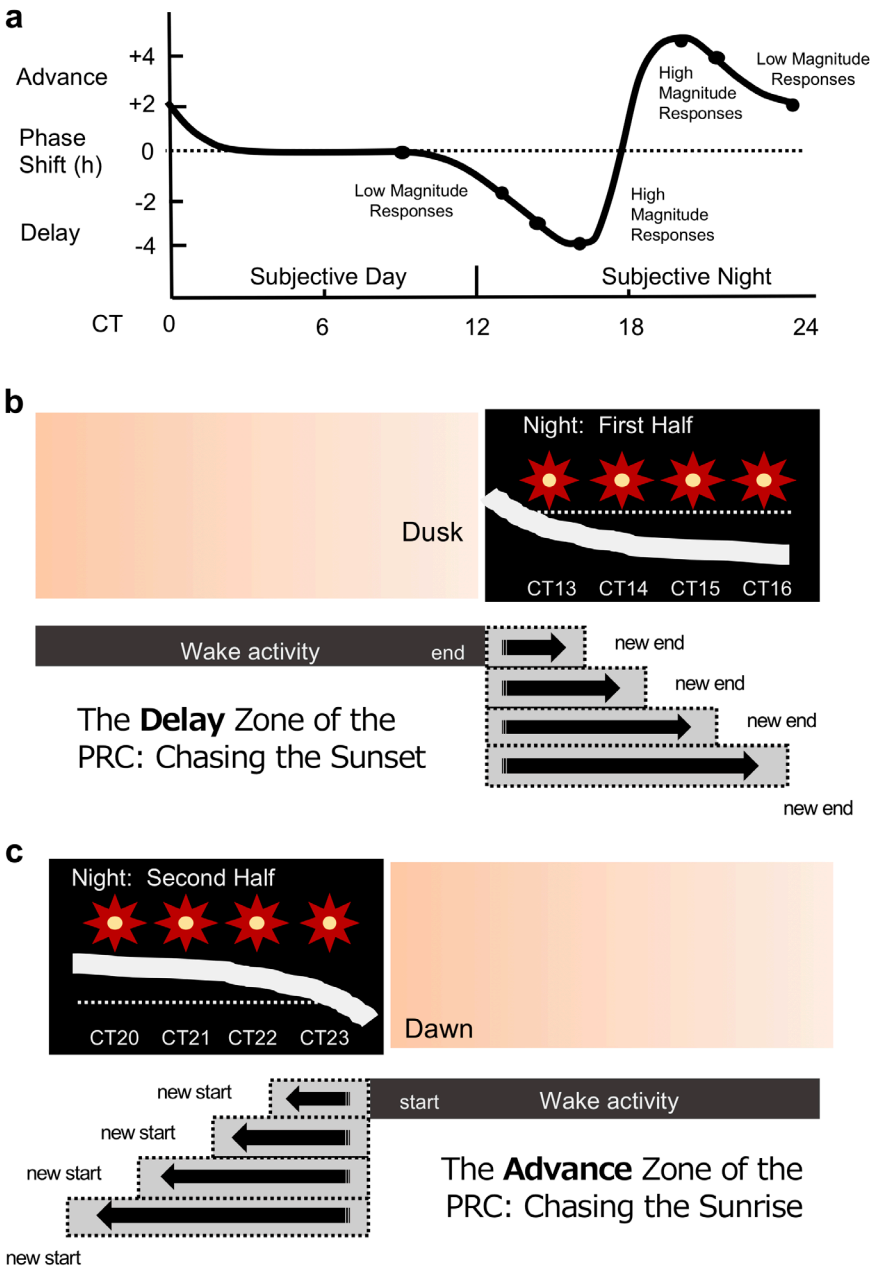

Figure 1. A canonical phase response curve to light. (a) Phase-shifts of physiological/behavioral rhythms that result from electric light exposure at night are typically charted with the magnitude of the shifts shown on the y-axis against the timing of light administration on the x-axis. Light's ability to trigger a circadian response is gated throughout the night, with particular pockets of sensitivity occurring several hours after dusk (**b**) or several hours before dawn (**c**). The pattern of these responses suggests that the pacemaker's photic sensitivity is scaled so that light exposure produces shifts that will always align the pacemaker's daily rhythm to the transitions of the light-dark cycle under which a person or animal is kept. Please note that not all human PRCs have a "dead zone" of photic insensitivity occurring during the subjective day. Depending on the stimulation protocol, some may take highly linear shapes, with a constant negative slope traveling between the advance and delay regions [73]. Figure adapted from [70].

that are registered by the pacemaker: the brighter or longer the pulse, the greater the resulting phase jump one should see up to some pseudosaturation level [36]. The implications of the reciprocity hypothesis for how the pacemaker uses sunlight in the real world to orient circadian timekeeping have never been directly articulated. Perhaps a safe assumption would be that the mere presence of light is enough to convince the pacemaker that the timing of dawn and dusk has migrated and that the size of this "bet" scales with irradiance and perceived illumination. Albeit simplistic, the general principle of reciprocity appears to hold when conventional electric lighting is shown to animals for periods exceeding 5 minutes up to about an hour. That is, the pacemaker will integrate broadspectrum input the same way over this span such that different trains of non-saturating pulses from ~5-60 min will elicit the same final phase shift as long as the overall photon flux is conserved [36-39]. Once a saturation point is hit, whether it be from a short, bright pulse or a long, dim one, the rate at which the phase-shifting response increases with further stimulation approaches zero.

RECIPROCITY IN HUMANS

The proposition of testing the reciprocity hypothesis in humans is complicated by the logistics of maintaining people under free-running conditions and tracking biomarkers closely associated with the endogenous phase of the pacemaker (most commonly body temperature or dim-light melatonin onset, DLMO; [40,41]). For these reasons, investigators ordinarily don't have the opportunity to assess light-induced circadian responses at hourly intervals of the subjective night, but must depend on more general assessments of the delay and advance areas of the PRC using blunter, less precise light regimens (for exceptions, see [42,43]). Often but not always, there is less description of the exact PRC transition where the mode of circadian resetting changes from delay-to-advance. Previous data suggest that this crossover does tend to coincide with a person's core body temperature minimum (CBT_{min}) [44-46], which in most individuals occurs near the end of nighttime sleep around 4:00-5:00 in the morning (marked by a fulcrum in Figure 2a).

Within this framework, Charles Czeisler and colleagues from the human chronophysiology lab at Brigham and Women's Hospital have conducted what is arguably the longest-running series of experiments to quantify the effects of light and darkness on the human circadian system [22,23,47-50] (light protocols are illustrated in Figure 2, panels b-g; the results from these protocols are enumerated in the Table). During the late biological night, the research team exposed cohorts of healthy volunteers to 5 h of white fluorescent light centered 1.5 h after the CBT_{min} for 3 consecutive days. The magnitude of pacemaker advances to different light levels ranging from 12-9500 lux in these 5-h blocks was measured (Figure 2b-d). Conversely, in the several hours of the early subjective night preceding the CBT_{min}, other cohorts were exposed to 6.5-h dim or bright light (0-9100 lux), or to different durations of 10,000 lux light, to evaluate how increasing photon count with brighter—as opposed to longer—pulses impacted delay resetting (Figure 2e-g). The results of these experiments spoke to several broad themes. First, in line with the reciprocity hypothesis, subjects that were exposed to higher illuminance in the early or late biological night showed greater resetting [22,23,47] (Figure 2b, c, and e; Table 1, rows 1-8 and 12-13). Both half-maximal phase-delaying and advancing responses were achieved with approximately 100 lux, with logistic curves best describing the relationship between light level and phase shift of the melatonin rhythm [22,23]. Second, longer (as opposed to briefer) pulses of the same illuminance were more effective phase-shifting agents in the early evening before CBT_{min}. Subjects shown 12 min of bright light exhibited significant 1-h delays in melatonin secretion, whereas those shown longer pulses (exceeding 2 h) had delays that were two-and-a half times larger [48] (Figure 2f; Table 1, rows 14-17). The duration-response relationship for light delivered in the early evening, like the illuminance-response relationship, could be modeled with high accuracy by a multi-parameter logistic function [22,48]. It is worth noting that the reciprocity trends documented by the Czeisler group are visible in the overall shape of the human PRC to broad spectrum fluorescent light that has been charted by several research teams. As the length of a 5,000-12,000-lux probe stimulus increases from 1 to 7 h, so does the peak-to-trough amplitude of the PRC (*i.e.*, summed height of the advance and delay regions) expand from about 2.2 h to 5.5 h [20,43-46].

RECIPROCITY IS NOT THE WHOLE STORY: THE EFFECTS OF LIGHT FRACTIONATION

A third feature of photic resetting that fell out of the Czeisler experimental series was the observation that, after a particular threshold of exposure, further introduction of light produced diminishing returns on phase movement (*i.e.*, the more the light was shown, the less the photic information got translated into the magnitude of the phase shift; Figure 3, insert) [47,48,51]. This meant that shorter durations of light, or dimmer pulses, had significantly greater resetting capacity per min of exposure or per lux invested than longer/brighter pulses. It also meant that the human pacemaker was most impressionable to information available at the beginning of a light stimulus (just as it was in non-human animals; [39]). The nonlinear duration-response function to light prompted Richard Kronauer, a colleague of Czeisler's, to develop a mathemat-

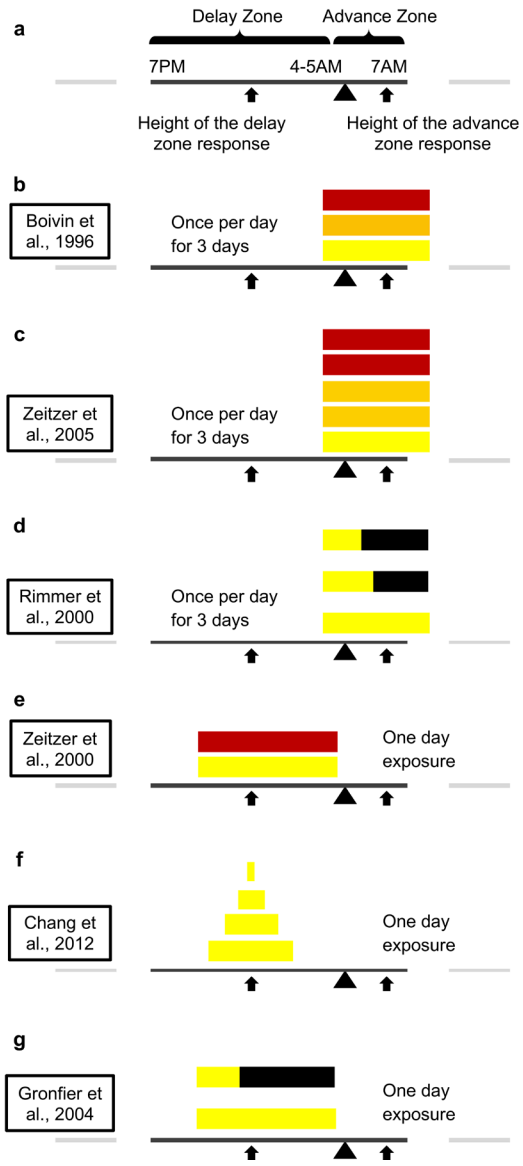

Figure 2. Minireview of human reciprocity experiments. (**a**) Schematic for the boundaries of the delay and advance zones in the human PRC to light. Coordinates revolve around the timing of a person's CBT_{min} (represented by black triangles; [44-46]). Evening light exposure prior to CBT_{min} delays the phase of the pacemaker rhythm; exposure after CBT_{min} phase advances it. Though human phase-shift experiments are ordinarily done under free-running conditions, for perspective, the delay and advance regions here are illustrated with respect to where they would occur under entrainment to the solar light-dark cycle. (**b-d**) Diagrams of phase-shifting experiments conducted in the pacemaker's advance zone. Light exposure is marked with rectangles (red = dim light; orange = medium amount of light; yellow = bright light; yellow/black = intermittent bright light protocol). Illuminance-response relationships for light-induced resetting of endogenous rhythms of body temperature or melatonin were standardized against a protocol where volunteers received a 5-h pulse centered 1.5 h after CBT_{min} on 3 consecutive days [23,47]. The effects of fragmenting the 5-h pulse into briefer episodes of intermittent light sandwiched between periods of complete darkness were examined for the brightest light condition (*i.e.*, 9500 lux, [49]). (**e-g**) Diagrams of phase-shifting experiments conducted in the pacemaker's delay zone. Illuminance and duration-response relationships for light-induced resetting of melatonin rhythms were standardized against a protocol where volunteers received a onetime 6.5-h pulse terminating about half-an-hour before CBT_{min} [22,48]. The effects of fragmenting the 6.5-h pulse into briefer episodes of light separated by darkness were examined for the brightest light condition (*i.e.*, 10,000 lux, [50]).

Table 1. Data from human reciprocity experiments.

	Stimulation Protocol	**Light Exposure**	**Physiology**
Reference	Description	Illuminance (eye level), Duration	Δ Phase
1 Boivin et al., 1996	Uniform stimulation, once per day for 3 consecutive days	180 lux, 5.0 hours	69.6 min
2 Boivin et al., 1996	Uniform stimulation, once per day for 3 consecutive days	1260 lux, 5.0 hours	161.4 min
3 Boivin et al., 1996	Uniform stimulation, once per day for 3 consecutive days	9500 lux, 5.0 hours	269.4 min
4 Zeitzer et al., 2005	Uniform stimulation, once per day for 3 consecutive days	12 lux, 5.0 hours	-41.4 min
5 Zeitzer et al., 2005	Uniform stimulation, once per day for 3 consecutive days	180 lux, 5.0 hours	108.0 min
6 Zeitzer et al., 2005	Uniform stimulation, once per day for 3 consecutive days	600 lux, 5.0 hours	225.0 min
7 Zeitzer et al., 2005	Uniform stimulation, once per day for 3 consecutive days	1260 lux, 5.0 hours	166.8 min
8 Zeitzer et al., 2005	Uniform stimulation, once per day for 3 consecutive days	9500 lux, 5.0 hours	259.8 min
9 Rimmer et al., 2000	5-min stimulation alt. with 20-min of rest for 5 hours (once per day for 3 consecutive days)	9500 lux, 1.6 hours	172.2 min
10 Rimmer et al., 2000	46-min stimulation alt. with 44-min of rest for 5 hours (once per day for 3 consecutive days)	9500 lux, 3.2 hours	234.0 min
11 Rimmer et al., 2000	Uniform stimulation, once per day for 3 consecutive days	9500 lux, 5.0 hours	271.2 min
12 Zeitzer et al., 2000	Uniform stimulation (one day of exposure)	106 lux, 6.5 hours	-108.0 min
13 Zeitzer et al., 2000	Uniform stimulation (one day of exposure)	9100 lux, 6.5 hours	-192.0 min
14 Chang et al., 2012	Uniform stimulation (one day of exposure)	10,000 lux, 0.2 hours	-64.2 min
15 Chang et al., 2012	Uniform stimulation (one day of exposure)	10,000 lux, 1.0 hour	-93.0 min
16 Chang et al., 2012	Uniform stimulation (one day of exposure)	10,000 lux, 2.5 hours	-137.4 min
17 Chang et al., 2012	Uniform stimulation (one day of exposure)	10,000 lux, 4.0 hours	-159.0 min
18 Gronfier et al., 2004	15-min stimulation alt. with 60-min of rest for 6.5 hours (one day of exposure)	10,000 lux, 1.5 hours	-140.4 min
19 Gronfier et al., 2004	Uniform stimulation (one day of exposure)	10,000 lux, 6.5 hours	-181.8 min

*Delays are listed with negative numbers, advances with positive numbers. See Figure 2, for an illustration of each protocol's timing.

ical model for how pacemaker photosensitivity changes throughout continued light exposure [52]. Broadly construed, the Kronauer model proposes that light stimulation always prompts an initial response by the pacemaker that persists in decaying fashion for a period of time after the stimulation has stopped (like the initial pedals of a bicycle wheel). In order to optimize phase-shifting drive, the onsets of the pulse must be long enough to reach full phase-shifting strength and then be balanced with periods of darkness so that steady activation of the pacemaker can occur without triggering competing processes that curb photosensitivity [52,53]. Implicit in this model is the general hypothesis that continuous light exposure can never reach the phase-shifting efficiency achieved with intermittent pulses when the pattern of intermittent exposure has been specifically tailored to match the rates with which the pacemaker build-ups and exhausts the responses set in motion by light. If true, the proposition would overturn the notion that reciprocity is the chief mechanism used by the pacemaker to compute the size of a phase shift.

The Czeisler group tested these assumptions by adapting their late and early subjective-night light administration protocols [49,50]. In the advance region, volunteers were given 3 consecutive days of 5-h white fluorescent light that was broken up into 5-min ramping pulses with 20 min of intervening darkness (31 percent duty cycle), or 46-min ramping pulses with 44 min of intervening darkness (63 percent duty cycle; Figure 2d; Table 1, rows 9-10) [49]. In the delay region, a separate cohort was given a 6.5-h regimen where 15-min blocks of light alternated with 60 min of darkness (amounting to a 23 percent duty cycle; Figure 2g; Table 1, row 18) [50]. Ostensibly, intermittent light exposure was nearly as effective a regimen as constant light in eliciting circadian responses. A 63 percent duty cycle during the late biological night preserved almost 90 percent of median resetting (Table 1, rows 9-11), while a 23 percent duty cycle during the early biological night preserved about 80 percent of it (Table 1, rows 18-19) [49,50]. However, a closer look at these numbers would suggest more modest gains. The investigators, possibly owing to the novelty of their study, did not consider the possibility that the total light exposure accumulated over fragmented stimulation was already closing in on levels that would saturate phase-shifting. The insert in Figure 3 plots the phase-shifting drive achieved in the delay and advance zones with the intermittent versus continuous protocols as a function of total light exposure (expressed in units of time-integrated illuminance or luminous exposure, lux • hour). Consistent with previous animal and human data, the reset efficacy of both types of protocols decayed exponentially with increasing amounts of light. Against this backdrop, one might expect that intermittent photic stimulation would counter the trend and achieve a reset efficacy per quanta higher-than-expected for the amount of light that was delivered. This was the case for the 23 percent duty cycle administered over the early biological night (*i.e.*, in the delay zone; Figure 3 insert, left panel, gray data point), but was not obvious for either of the fractionation strategies used in the late evening (*i.e.*, in the advance zone; Figure 3 insert, right panel, gray data points). The larger takeaway from the Czeisler group's exploration of the Kronauer model is a positive one: in principle, light fractionation is a strategy that can be used to communicate more efficiently with the pacemaker. Nevertheless, the length of the pulses (*e.g.*, 15 min vs. shorter blocks) and the breaks between them require finer calibration to balance the rate constants that Kronauer described.

Other characterizations of intermittent light and its potential to treat circadian disorder have been spearheaded by Rush University's Biological Rhythms Research Laboratory in another series of studies led by Charmane Eastman and Helen Burgess (elegant applications can be found in [42,54-56]). The Rush group's studies have not shown the same continuity as those reported by Czeisler *et al.*, who have historically used the same lighting equipment to evaluate the circadian responses generated by several interrelated stimulation protocols. However, the Rush group's varied approach has allowed them to explore other aspects of intermittent light administration, including constructing the first PRC to patterned exposure to blue LED light (three 30-min pulses delivered over 2 hours at ~185 lux, once a day for 3 days; [42]) and the first adolescent PRC to bright fluorescent (5000 K) light intermixed with periods of ambient room lighting (4100 K) [55]. These experiments have been pivotal towards demonstrating the real-world applicability of light fractionation from a commercially available LED unit as a countermeasure to sleep-circadian disruption and have provided some insight into how phototherapy might be practiced in a population naturally vulnerable to circadian misalignment (*i.e.*, teenagers, whose delayed chronotype is at odds with early school start times).

Buoyed by insights that were first made in wild bats and lab rodents [57-61], light fractionation in circadian phase-shifting is now being surveyed in humans at several finer temporal resolutions, from milliseconds to seconds [51,62-67]. As might be predicted from Kronauer-esque drive models, the circadian effects of intermittent flashes delivered in the millisecond range do not taper off with repeated application and possess significantly more resetting capacity than uninterrupted stretches of light that last an hour, a minute, or just a few seconds [61,67-69]. How much more capacity? The phase-shifting drive created by millisecond photic exposure is sufficiently powerful that it can't be visualized properly on the same reset efficacy

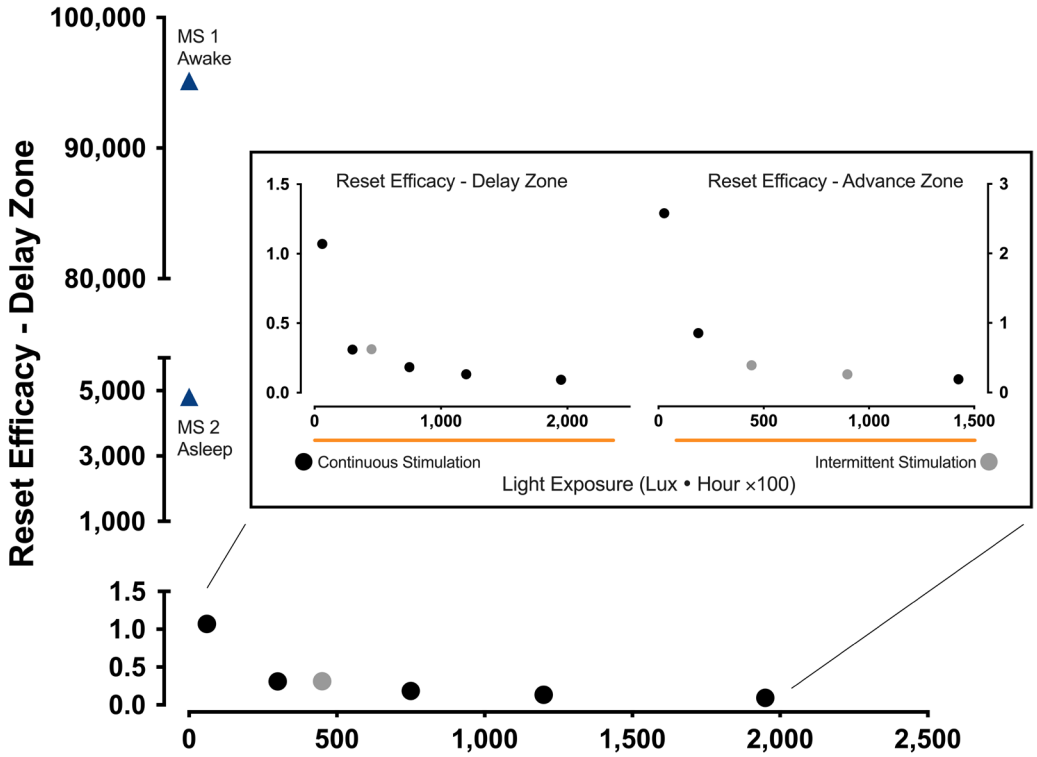

Figure 3. Pacemaker responses to intermittent versus continuous light. (***Insert***) The efficiency with which continuous or intermittent light phase-shifts the human body temperature or melatonin rhythm is plotted as a function of total light exposure. Reset efficacy in the delay and advance zones is calculated by dividing the size of the shift observed (in minutes) by the amount of light used to produce it (in units of lux-hours × 100). Data in the insert are derived from the mean or median phase-shift values reported in [47-50] and summarized in Table 1, rows 1-3, 9-11, and 14-19. (***Larger graph***) The *delay zone* data in the insert is rescaled within the larger graph to accommodate the scales-of-magnitude difference in reset efficacy between hour-long and millisecond stimuli. Millisecond data, plotted with blue triangles, are derived from the average phase-shift values reported for individuals exposed to 2-msec flashes of xenon light (473-2995 lux) delivered once every 30-60s for an hour. Subjects were administered these flash sequences while awake [65] or asleep [66]. These regimens produced 30-45 min shifts of the melatonin rhythm.

scale used to plot the efficacy of minute and hour-long light administration (Figure 3, zoom-out plot, triangle data points; reference data from [65,66]). The scope of this effect suggests that the pacemaker employs an additional layer of light-integration logic far removed from reciprocity and that this logic can't be completely understood even with present-day drive models [70].

LIGHT FRACTIONATION IN PHOTOTHERAPY: CLOSING THOUGHTS

The circadian system depends on external photoreceptors to filter the flow of stimulus information available at twilight in order to compute highly specific time-of-day estimates. Reading the progression of wavelength and irradiance changes that envelop dawn and dusk, the photoreceptors evolved to reliably calibrate the pacemaker's internally created day and night so that the length of the two together matched the length of the solar cycle in some given phase alignment relative to sunrise and sunset [24,71]. While this interplay between photoreceptor and clock has been appreciated for at least two decades [72], the reading frame that is created as light is sampled from the environment and processed through the retinohypothalamic tract and SCN circuitry has not been critically evaluated save for the idea that light must be integrated over long windows of exposure—and in an unbiased fashion photon for photon—to ensure tightly synchronized photoentrainment [39]. Emergent literature on the circadian phase-shifting effects of intermittent light, including that concerning millisecond flashes, now suggests *prima facie* that this reading frame is not continuous: instead, photic information is preferentially integrated one fragment or "syllable" at a time to generate a coherent entrainment response. Speculation is merited as to why this might be the case.

One possible answer is hinted at if one considers the sensory information the pacemaker monitors to keep the

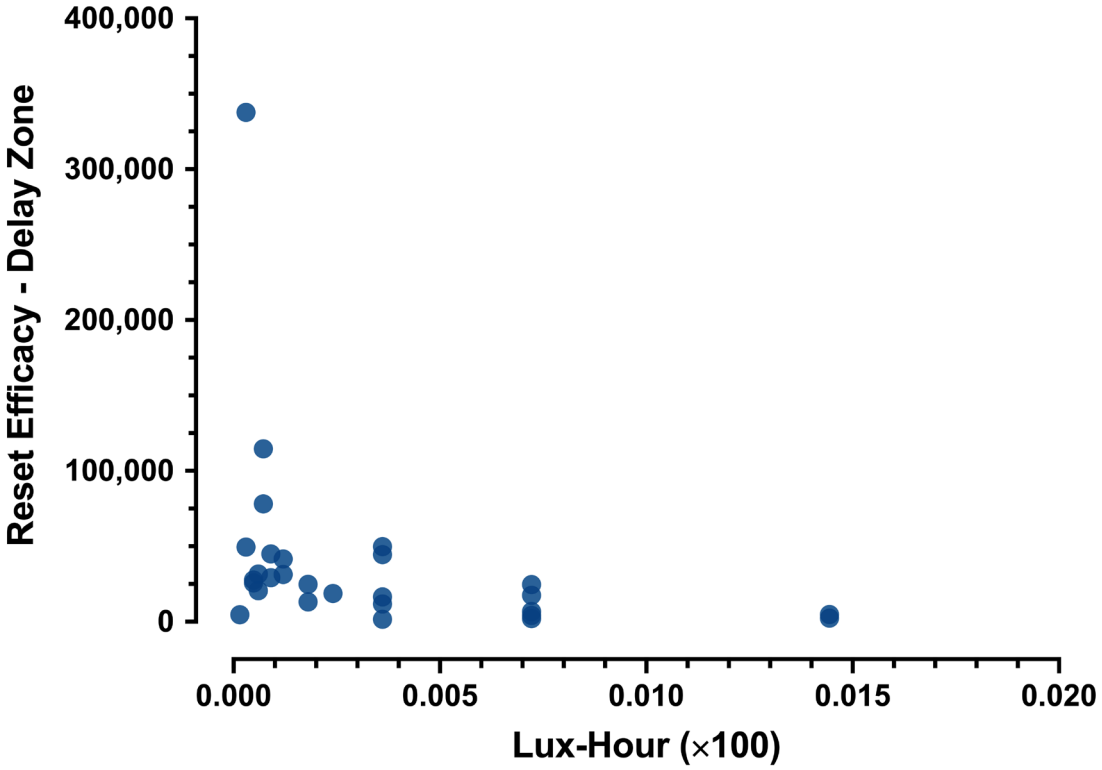

Figure 4. Pacemaker responses to flash sequences with different interstimulus intervals. The efficiency with which flash stimulation (xenon, 1700-1805 lux) phase-shifts the human melatonin rhythm is plotted as a function of total light exposure. Data are taken from 29/31 individuals who showed significant delays to flash sequences where the interstimulus interval varied between 2.5–240 s. Delays as large as 1.5-3.0 h were observed in the sample [67]. The arrangement of the scatter plot suggests that the phase-shifting drive of sequential millisecond-flash exposure follows similar decay kinetics as continuous minute or hour-long light administration, raising the possibility that drive is integrated by the pacemaker independently across different timescales (as articulated in [68]).

body entrained to sunlight. In short, the pacemaker is trying to locate dawn and dusk, twilight sequences where light levels steadily (and then precipitously) increase or decrease and where the progression of these illumination changes is pegged to changes in the spectrum of incident light. When this concept is merged with the empirical data collected from light fractionation experiments, the two together suggest that there are multiple layers of temporal integration (threads) that go into pacemaker's time-of-day estimates and that in this dynamic reading frame the pacemaker orients to *combined fluctuations* in light intensity, spectrum, duration, and probability of overlapping exposures. To track the movement of the sun, the pacemaker can resort to photon detection (irradiance); however, the precision of this tracking in nature is dependent on the inclusion of light characteristics such as color and on discrete episodes of light that are expected to follow the incremental flow of twilight at dawn or dusk [8]. What this perspective also raises is the prospect of artificially inserting information into the pacemaker's reading frame for phototherapeutic application. If the photic information that is guiding the pacemaker's phase response is naturally synthesized one syllable at a time before a coherent phase jump is triggered, then it is theoretically possible to modify each of those syllables along several parameters to refine the response so that it suits a particular individual or condition. With the commercialization of LEDs, devices which emit narrowband light with highly precise temporal control, future phototherapy protocols will be able to change stimulus parameters from one syllable to the next in a way that could not have been previously envisioned. Across milliseconds, light regimens can be assembled that specify particular sequences of flash exposure that morph in series, modifying parameters such as illuminance (*e.g.*, 0–100 lx), pulse shape (*e.g.*, patterns of intensity modulation within a pulse), width (*e.g.*, milliseconds to seconds), color (visible spectrum, ~380-760 nm), UVA content (non-toxic part of UVA radiation spectrum, 365 nm), or interstimulus interval (temporal distance between syllables). When properly considered, the breadth of this parameter space is actually quite large. Nevertheless, if recent work is any indication,

we will—eventually—have to grapple with this complexity. By just modifying the interstimulus interval between the millisecond flashes in their hour-long light protocols (incorporating as few as 30 flashes), Najjar and Zeitzer showed that fractionation can not only increase the reset efficacy of light exposure per quanta but—in an absolute sense—produce larger delay shifts in people than those produced with 1 h of continuous stimulation with equiluminous light (this, despite the dramatic 3800× difference in exposure; see Figure 4 for a reproduction of data from 29 individuals [67], with reset efficacy plotted according to total illuminance). This finding poses a serious challenge to our current understanding of light's effects on the human circadian system and opens a new field of inquiry regarding how high-speed administration of LED pulses can be deployed in the clinic to treat disorders. We will lose a valuable opportunity to help more patients if we don't begin to consider the novel biological effects that might be afforded by newer precision light emission technologies such as LED lighting.

Acknowledgments: Financial support to the author was provided by Science Foundation Arizona (SFAz) and the BIO5 Institute at the University of Arizona. S. Kaladchibachi is acknowledged for providing a critical read of the text.

REFERENCES

1. Golombek DA, Rosenstein RE. Physiology of circadian entrainment. Physiol Rev. 2010 Jul;90(3):1063-102.
2. Edgar RS, Green EW, Zhao Y, van Ooijen G, Olmedo M, Qin X, Xu Y, Pan M, Valekunja UK, Feeney KA, Maywood ES, Hastings MH, Baliga NS, Merrow M, Millar AJ, Johnson CH, Kyriacou CP, O'Neill JS, Reddy AB. Peroxiredoxins are conserved markers of circadian rhythms. Nature. 2012 May 16;485(7399):459-64.
3. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. Science. 2002 Feb 8;295(5557):1070-3.
4. Freedman MS, Lucas RJ, Soni B, von Schantz M, Muñoz M, David-Gray Z, Foster R. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science. 1999 Apr 16;284(5413):502-4.
5. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. Nat Neurosci. 2001;4(12):1165.
6. Hughes S, Jagannath A, Hankins MW, Foster RG, Peirson SN. Photic regulation of clock systems. Methods Enzymol. 2015;552:125-43.
7. Lucas RJ, Lall GS, Allen AE, Brown TM. How rod, cone, and melanopsin photoreceptors come together to enlighten the mammalian circadian clock. Prog Brain Res. 2012;199:1-18.
8. Walmsley L, Hanna L, Mouland J, Martial F, West A, Smedley AR, Bechtold DA, Webb AR, Lucas RJ, Brown TM. Colour as a signal for entraining the mammalian circadian clock. PLoS Biol. 2015 Apr 17;13(4):e1002127.
9. Czeisler CA, Richardson GS, Zimmerman JC, Moore-Ede MC, Weitzman ED. Entrainment of human circadian rhythms by light-dark cycles: a reassessment. Photochem Photobiol. 1981 Aug;34(2):239-47.
10. Bartness TJ, Song CK, Demas GE. SCN efferents to peripheral tissues: implications for biological rhythms. J Biol Rhythms. 2001 Jun;16(3):196-204.
11. Evans JA, Suen TC, Callif BL, Mitchell AS, Castanon-Cervantes O, Baker KM, et al. Shell neurons of the master circadian clock coordinate the phase of tissue clocks throughout the brain and body. BMC Biology. 2015 Dec;13(1):43.
12. LeSauter J, Silver R. Output signals of the SCN. Chronobiol Int. 1998 Jan 1;15(5):535-50.
13. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol. 2010;72:517-49.
14. Longo VD, Panda S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Cell Metab. 2016 Jun 14;23(6):1048-1059.
15. Johnson CH. Forty years of PRCs--what have we learned? Chronobiol Int. 1999 Nov;16(6):711-43.
16. Daan S, Pittendrigh C. A functional analysis of circadian pacemakers in nocturnal rodents: II. The variability of phase response curves. J Comp Physiol A. 1976;106:253-66.
17. Pittendrigh, CS. On temporal organization in living systems. Harvey Lectures 1961; 56:93-125.
18. Horton TH. Conceptual issues in the ecology and evolution of circadian rhythms. In Circadian Clocks 2001 (pp. 45-57). Springer, Boston, MA.
19. Hall JC, Rosbash M. Genes and biological rhythms. Trends Genet. 1987 Jan 1;3:185-91.
20. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. A phase response curve to single bright light pulses in human subjects. J Physiol. 2003 Jun 15;549(Pt 3):945-52.
21. Schwartz WJ, Zimmerman P. Circadian timekeeping in BALB/c and C57BL/6 inbred mouse strains. J Neurosci. 1990 Nov;10(11):3685-94.
22. Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol. 2000 Aug 1;526 Pt 3:695-702.
23. Zeitzer JM, Khalsa SB, Boivin DB, Duffy JF, Shanahan TL, Kronauer RE, Czeisler CA. Temporal dynamics of late-night photic stimulation of the human circadian timing system. Am J Physiol Regul Integr Comp Physiol. 2005 Sep;289(3):R839-44.
24. Foster RG, Helfrich-Förster C. The regulation of circadian clocks by light in fruitflies and mice. Philos Trans R Soc Lond B Biol Sci. 2001 Nov 29;356(1415):1779.
25. Hall JC, Rosbash M. Genetic and molecular analysis of biological rhythms. J Biol Rhythms. 1987 Fall;2(3):153-78.
26. Konopka RJ, Benzer S. Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci USA. 1971 Sep;68(9):2112-6.
27. Young MW, Kay SA. Time zones: a comparative genetics of circadian clocks. Nat Rev Genet. 2001 Sep;2(9):702-15.
28. DeCoursey PJ. Daily light sensitivity rhythm in a rodent. Science. 1960 Jan 1;131(3392):33-5.
29. Gooley JJ. Light resetting and entrainment of human circadian rhythms. In Biological Timekeeping: Clocks,

Rhythms and Behaviour 2017 (pp. 297-313). Springer, New Delhi.
30. Gooley JJ, Rajaratnam SM, Brainard GC, Kronauer RE, Czeisler CA, Lockley SW. Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. Sci Transl Med. 2010 May 12;2(31):31ra33.
31. Rüger M, St Hilaire MA, Brainard GC, Khalsa SB, Kronauer RE, Czeisler CA, Lockley SW. Human phase response curve to a single 6.5 h pulse of short-wavelength light. J Physiol. 2013 Jan 1;591(1):353-63.
32. Chang AM, Scheer FA, Czeisler CA. The human circadian system adapts to prior photic history. J Physiol. 2011 Mar 1;589(Pt 5):1095-102.
33. Refinetti R. Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. Chronobiol Int. 2003 May;20(3):417-40.
34. Mure LS, Cornut PL, Rieux C, Drouyer E, Denis P, Gronfier C, Cooper HM. Melanopsin bistability: a fly's eye technology in the human retina. PLoS One. 2009 Jun 24;4(6):e5991.
35. Mure LS, Rieux C, Hattar S, Cooper HM. Melanopsin-dependent nonvisual responses: evidence for photopigment bistability in vivo. J Biol Rhythms. 2007 Oct;22(5):411-24.
36. Takahashi JS, DeCoursey PJ, Bauman L, Menaker M. Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature. 1984 Mar 8-14;308(5955):186-8.
37. Best JD, Maywood ES, Smith KL, Hastings MH. Rapid resetting of the mammalian circadian clock. J Neurosci. 1999 Jan 15;19(2):828-35.
38. Dkhissi-Benyahya O, Sicard B, Cooper HM. Effects of irradiance and stimulus duration on early gene expression (Fos) in the suprachiasmatic nucleus: temporal summation and reciprocity. J Neurosci. 2000 Oct 15;20(20):7790-7.
39. Nelson DE, Takahashi JS. Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (Mesocricetus auratus). J Physiol. 1991 Aug;439:115-45.
40. Pandi-Perumal SR, Smits M, Spence W, Srinivasan V, Cardinali DP, Lowe AD, Kayumov L. Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2007 Jan 30;31(1):1-11.
41. Waterhouse J, Drust B, Weinert D, Edwards B, Gregson W, Atkinson G, Kao S, Aizawa S, Reilly T. The circadian rhythm of core temperature: origin and some implications for exercise performance. Chronobiol Int. 2005;22(2):207-25.
42. Revell VL, Molina TA, Eastman CI. Human phase response curve to intermittent blue light using a commercially available device. J Physiol. 2012 Oct 1;590(19):4859-68.
43. St Hilaire MA, Gooley JJ, Khalsa SB, Kronauer RE, Czeisler CA, Lockley SW. Human phase response curve to a 1 h pulse of bright white light. J Physiol. 2012 Jul 1;590(13):3035-45.
44. Dawson D, Lack L, Morris M. Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. Chronobiol Int. 1993 Apr;10(2):94-102.
45. Minors DS, Waterhouse JM, Wirz-Justice A. A human phase-response curve to light. Neurosci Lett. 1991 Nov 25;133(1):36-40.
46. Van Cauter E, Sturis J, Byrne MM, Blackman JD, Leproult R, Ofek G, L'Hermite-Balériaux M, Refetoff S, Turek FW, Van Reeth O. Demonstration of rapid light-induced advances and delays of the human circadian clock using hormonal phase markers. Am J Physiol. 1994 Jun;266(6 Pt 1):E953-63.
47. Boivin DB, Duffy JF, Kronauer RE, Czeisler CA. Dose-response relationships for resetting of human circadian clock by light. Nature. 1996 Feb 8;379(6565):540-2.
48. Chang AM, Santhi N, St Hilaire M, Gronfier C, Bradstreet DS, Duffy JF, Lockley SW, Kronauer RE, Czeisler CA. Human responses to bright light of different durations. J Physiol. 2012 Jul 1;590(13):3103-12.
49. Rimmer DW, Boivin DB, Shanahan TL, Kronauer RE, Duffy JF, Czeisler CA. Dynamic resetting of the human circadian pacemaker by intermittent bright light. Am J Physiol Regul Integr Comp Physiol. 2000 Nov;279(5):R1574-9.
50. Gronfier C, Wright KP Jr, Kronauer RE, Jewett ME, Czeisler CA. Efficacy of a single sequence of intermittent bright light pulses for delaying circadian phase in humans. Am J Physiol Endocrinol Metab. 2004 Jul;287(1):E174-81.
51. Rahman SA, St Hilaire MA, Chang AM, Santhi N, Duffy JF, Kronauer RE, Czeisler CA, Lockley SW, Klerman EB. Circadian phase resetting by a single short-duration light exposure. JCI Insight. 2017 Apr 6;2(7):e89494.
52. Kronauer RE, Forger DB, Jewett ME. Quantifying human circadian pacemaker response to brief, extended, and repeated light stimuli over the phototopic range. J Biol Rhythms. 1999 Dec;14(6):500-15.
53. Jewett ME, Forger DB, Kronauer RE. Revised limit cycle oscillator model of human circadian pacemaker. J Biol Rhythms. 1999 Dec;14(6):493-9.
54. Burgess HJ, Crowley SJ, Gazda CJ, Fogg LF, Eastman CI. Preflight adjustment to eastward travel: 3 days of advancing sleep with and without morning bright light. J Biol Rhythms. 2003 Aug;18(4):318-28.
55. Crowley SJ, Eastman CI. Human Adolescent Phase Response Curves to Bright White Light. J Biol Rhythms. 2017 Aug;32(4):334-344.
56. Smith MR, Cullnan EE, Eastman CI. Shaping the light/dark pattern for circadian adaptation to night shift work. Physiol Behav. 2008 Oct 20;95(3):449-56.
57. Arvanitogiannis A, Amir S. Resetting the rat circadian clock by ultra-short light flashes. Neurosci Lett. 1999 Feb 19;261(3):159-62.
58. Joshi D, Chandrashekaran MK. Bright light flashes of 0.5 milliseconds reset the circadian clock of a microchiropteran bat. J Exp Zool. 1984 May;230(2):325-8.
59. Joshi D, Chandrashekaran MK. Light flashes of different durations (0.063-3.33 msec) phase shift the circadian flight activity of a bat. J Exp Zool. 1985 Feb;233(2):187-92.
60. Van Den Pol AN, Cao V, Heller HC. Circadian system of mice integrates brief light stimuli. Am J Physiol. 1998 Aug;275(2 Pt 2):R654-7.
61. Vidal L, Morin LP. Absence of normal photic integration in the circadian visual system: response to millisecond light flashes. J Neurosci. 2007 Mar 28;27(13):3375-82.
62. Figueiro MG. Individually tailored light intervention through closed eyelids to promote circadian alignment and

sleep health. Sleep Health. 2015 Mar 1;1(1):75-82.
63. Figueiro MG, Bierman A, Rea MS. A train of blue light pulses delivered through closed eyelids suppresses melatonin and phase shifts the human circadian system. Nat Sci Sleep. 2013 Oct 4;5:133-41.
64. Figueiro MG, Plitnick B, Rea MS. Pulsing blue light through closed eyelids: effects on acute melatonin suppression and phase shifting of dim light melatonin onset. Nat Sci Sleep. 2014 Dec 2;6:149-56.
65. Zeitzer JM, Ruby NF, Fisicaro RA, Heller HC. Response of the human circadian system to millisecond flashes of light. PLoS One. 2011;6(7):e22078.
66. Zeitzer JM, Fisicaro RA, Ruby NF, Heller HC. Millisecond flashes of light phase delay the human circadian clock during sleep. J Biol Rhythms. 2014 Oct;29(5):370-6.
67. Najjar RP, Zeitzer JM. Temporal integration of light flashes by the human circadian system. J Clin Invest. 2016 Mar 1;126(3):938-47.
68. Kaladchibachi S, Negelspach DC, Fernandez F. Circadian phase-shifting by light: Beyond photons. Neurobiol Sleep Circadian Rhythms. 2018 Jun 1;5:8-14.
69. Negelspach DC, Kaladchibachi S, Fernandez F. The circadian activity rhythm is reset by nanowatt pulses of ultraviolet light. Proc Biol Sci. 2018 Aug 1;285(1884).
70. Kaladchibachi S, Fernandez F. Precision light for the treatment of psychiatric disorders. Neural Plast. 2018 Jan 11;2018:5868570.
71. Wehr TA, Aeschbach D, Duncan Jr WC. Evidence for a biological dawn and dusk in the human circadian timing system. J Physiol. 2001 Sep;535(3):937-51.
72. Roenneberg T, Foster RG. Twilight times: light and the circadian system. Photochem Photobiol. 1997 Nov;66(5):549-61.
73. Jewett ME, Rimmer DW, Duffy JF, Klerman EB, Kronauer RE, Czeisler CA. Human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients. Am J Physiol Regul Integr Comp Physiol. 1997 Nov;273(5):R1800-9.