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An exploration of the temporal dynamics of circadian resetting responses to short and long duration light exposures::

Cross-species consistencies and differences

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Abstract

Light is the most effective environmental stimulus for shifting the mammalian circadian pacemaker. Numerous studies have been conducted across multiple species to delineate wavelength, intensity, duration, and timing contributions to the response of the circadian pacemaker to light. Recent studies have revealed a surprising sensitivity of the human circadian pacemaker to short pulses of light. Such responses challenged “photon counting” based theories of the temporal dynamics of the mammalian circadian system to both short and long duration light stimuli. Here we collate published light exposure data from multiple species, including gerbil, hamster, mouse, and human, to investigate these temporal dynamics, and explore how the

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Author Contributions

REK, MSH, SAR, CAC, and EBK performed data analysis and interpretation and prepared manuscript.

Conflicts of interest

None of the authors have conflicts of interest directly related to the work presented in this manuscript. In the interest of full disclosure, we report the following relationships: REK has no conflicts to report. MSH has consulted for The MathWorks, Inc. and received travel support from the Mayo Clinic Metabolomics Resource Core. SAR holds patents for prevention of circadian rhythm disruption by using optical filters and improving sleep performance in subjects exposed to light at night; SAR owns equity in Melcort Inc.; SAR is a co-investigator on studies sponsored by Biological Illuminations, LLC; Vanda Pharmaceuticals Inc. SAR has provided paid consulting services to Sultan & Knight Limited t/a Circadia. CAC has received education/research support to BWH from Cephalon Inc., Mary Ann & Stanley Snider via Combined Jewish Philanthropies, National Football League Charities, Optum, Philips Respironics, Inc., ResMed Foundation, San Francisco Bar Pilots, Schneider Inc., Sysco, Cephalon, Inc, Jazz Pharmaceuticals, Takeda Pharmaceuticals, Teva Pharmaceuticals Industries, Ltd, Sanofi-Aventis, Inc, Sepracor, Inc, and Wake Up Narcolepsy. CAC has received consulting fees and/or served as a paid member of scientific advisory board for Bose Corporation, Boston Celtics, Boston Red Sox, Columbia River Bar Pilots, Institute of Digital Media and Child Development, Klarman Family Foundation, Samsung Electronics, Quest Diagnostics, Inc, Vanda Pharmaceuticals, Zurich Insurance Company, Ltd, Purdue Pharma, LP, McGraw Hill, Houghton Mifflin Harcourt/Penguin, Koninklijke Philips Electronics, N.V., Cephalon, Inc, Washington State Board of Pilotage Commissioners, and GanESCO Inc. CAC owns equity interest in Vanda Pharmaceuticals. CAC holds a number of process patents in the field of sleep/circadian rhythms (e.g., photic resetting of the human circadian pacemaker). These are available from BWH upon request. CAC has also served as an expert on various legal and technical cases related to sleep and/or circadian rhythms including those involving the following commercial entities: Complete General Construction Company, FedEx, Greyhound, HG Energy LLC, South Carolina Central Railroad Co., Steel Warehouse Inc., Stric-Lan Companies LLC, Texas Premier Resource LLC and United Parcel Service (UPS). CAC's interests were reviewed and managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies. EBK has consulted for Pfizer, Inc. and received travel support from the Sleep Research Society and the National Sleep Foundation.

circadian system integrates light information at both short and long duration time scales to produce phase shifts. Based on our investigation of these datasets, we propose three new interpretations: (1) intensity and duration are independent factors for total phase shift magnitude; (2) the possibility of a linear/log temporal function of light duration that is universal for all intensities for durations less than approximately 800 seconds; and (3) a potential universal minimum light duration of ~0.7 sec that describes a “dead zone” of light stimulus. We show that these properties appear to be consistent across mammalian species. These interpretations, if confirmed by further experiments, have important practical implications in terms of understanding the underlying physiology and for the design of lighting regimens to reset the mammalian circadian pacemaker.

Keywords

circadian pacemaker; duration response; light intensity; mathematical modeling; photic integration

Introduction

The phase of the mammalian circadian pacemaker can be shifted by environmental light. The size of the phase shift depends on the wavelength, intensity, duration, and timing of the light stimulus (Zeitzer et al., 2000; Khalsa et al., 2003; Lockley et al., 2003; Gooley et al., 2010; Chang et al., 2012; St Hilaire et al., 2012; Rüger et al., 2013; Rahman et al., 2017); Nelson and Takahashi, 1991; Comas et al., 2006; Dkhissi-Benyahya et al., 2007; Czeisler, 1995; Czeisler and Gooley, 2007). These responses have been extensively studied in both nocturnal and diurnal mammals, including humans. Understanding these responses is crucial for basic science and translation to applications. We here focus on light intensity and duration factors that influence these phase shifts by analyzing results from multiple mammalian species.

The circadian phase resetting system has often been assumed to act like a “photon counter” (Nelson and Takahashi, 1991; Lall et al., 2010; Brown, 2016) that predicts linear increasing phase shift with increasing photons (from either intensity or duration). This assumption, however, is not consistent with observations in multiple species (Kronauer et al., 1999; Vidal and Morin, 2007; Zeitzer et al., 2011; Najjar and Zeitzer, 2016). For example, we recently published two papers (Chang et al., 2012; Rahman et al., 2017) summarizing the temporal dynamics of human delay phase shifts achieved for a range of light exposure durations, T , extending from 15 seconds to 6.5 hours that show that the response is not linear, as would be expected for a system that counts photons. These results prompted us to analyze data to assess the temporal dynamics of photic resetting responses from multiple species.

The particular data sets on which we focus in the upcoming sections were chosen based on two factors (1) responses were elicited by at least three different light stimulus durations and (2) data could be extracted directly from the publications (i.e., from figures or tables). Four data sets across four different mammalian species met our criteria. We begin our discussion with delay phase shifting in humans (Section 2) and c-fos induction in gerbils (Section 3) in response to different light durations to introduce our mathematical formulation for short-duration light stimuli (Section 4). We then discuss the interaction between light intensity and

duration in golden hamsters (Section 5) and phase-shifting in mice (Section 6) before introducing a formal model (Section 7).

Human phase-resetting responses to light: differences between short and long-duration exposures

Our human phase delay resetting data are summarized in Table 1 (Gronfier et al., 2004; Chang et al., 2012; Rahman et al., 2017). Targeted corneal illumination for all stimulus durations was ~9,500 lux ($>8 \times 10^6 \mu\text{W}/\text{cm}^2$) generated using ceiling-mounted 4100K fluorescent lamps. The first robust finding from this data set (Table 1 and Figure 1) is the monotonic decline of *yield* or *resetting rate* ($\frac{y}{T}$, minutes of phase delay shift per minute of light exposure) with increasing stimulus duration (T; note that T refers to stimulus duration, not time). Simply interpreted, for any light stimulus duration, it is the early photons that have the disproportionately largest effect. As the stimulus proceeds (i.e., longer duration), later photons have progressively less effect. We will now quantify this effect.

When these data are plotted (Figure 1A), the rate at which the average phase shift is increasing with longer stimulus durations (T, in minutes) appears different for the three shortest duration stimuli (T = 12 minutes) as compared to the three longest duration stimuli (T = 150 minutes), with the three longest duration stimuli appearing to be linear. We fit a linear regression through the resetting responses from the three longest duration stimuli, which results in an intercept (y_0) of 76.75 minutes and a slope of the resetting rate $M = \frac{dy}{dT}$ of 0.27 minutes of phase shift per minute of light exposure (adjusted $R^2 = 0.95$). The response elicited by T = 60 minutes to bright light is not included in the fit applied to either the short or long duration stimuli; in Section 7 we will discuss why T = 60 minutes is not included in these first calculations.

To explore the phase-resetting responses from the three shortest duration stimuli (T = 12 minutes), we choose a strongly nonlinear transformation of T: $x = \log T$, where the original unit of minutes has been converted to seconds (Figure 1B). The relationship between phase-shift and $x = \log T$ for the three longest duration stimuli now appears exponential and the relationship between phase-shift and $x = \log T$ for the three shortest duration stimuli appears to be linear (Figure 1B). This linear fit is expressed mathematically as:

$$y = m(x - x_0) \quad (1)$$

where $m = \frac{dy}{d \log T}$. Note that we use m for the short-T relationship and M for the long-T relationship.

We find $m = 23.13$ minutes and $x_0 = -0.19$. This value for x_0 translates into a stimulus duration $T_0 = 10^{x_0} = 0.65$ seconds ($n = 3$, adjusted R^2 of 0.95), which represents the response onset of the system. We now write the short T (light duration = 12 minutes) relationship in its primary form:

$$y = m(\log T - \log T_0) \quad (1a)$$

or equivalently:

$$y = m \log \left(\frac{T}{T_0} \right) \quad (1b)$$

[Note: we are using log (base 10) rather than ln (base e) here since the data are in log units. We convert to ln later for ease of calculations.] This relationship embodies only two parameters, m and T_0 . It has an elegant simplicity, and we refer to it as the linear/log function. Later in this paper, we will analyze data from several mammalian species (gerbil, hamster, and mouse) that suggest their responses to short duration light stimuli display this same linear/log functionality.

In Figure 1B, we now have two cases: linear/log for the three shortest duration stimuli and linear/linear for the three longest duration stimuli.

It is immediately apparent from Figure 1B that the two cases do not intersect, although they come close. We undertake to calculate the stimulus duration T^* at the closest approach. We observe that at the closest approach, the two curves must show the same $\frac{dy}{dx}$, and therefore the same $\frac{dy}{dT}$. For long T , $\frac{dy}{dT} = M$. For the three shortest duration stimuli, we have from Equation (1b) that

$$\frac{dy}{dT} = m \frac{d(\log T)}{dT} = m \frac{1}{\ln(10)T} = 23.13 \left(\frac{1}{2.303T} \right) = \frac{10.04}{T} = \frac{\tilde{m}}{T} \quad (1c)$$

(where $\ln 10 = 2.303$ when we convert from log to ln to analyze the derivative). Since in this equation T occurs in the denominators of both $\frac{dy}{dT}$ and $\frac{10.04}{T}$, we are free to assign to T whatever units we choose; here, we choose minutes. Equating the derivatives $M = 0.27 = \frac{10.04}{T}$ (where 0.27 is derived from long T stimuli and 10.04 is derived from short T stimuli) gives $T^* = \frac{10.04}{0.27} = 37.19$ minutes and $\ln T^* = 3.62$.

At this point, we address several fundamental concepts. In the immediately preceding paragraph, we treated the linear/log and linear/linear cases as mathematical equals. One goal of this paper is to show how the linear/log *function*, which is presented formally below in Equation 3, is encountered across mammalian species; how it works for both advance and delay phase shifts; how it functions independently of the intensity of the light stimulus; and how time-dependence is embodied in the single parameter T_0 , which shows remarkable consistency across species. In contrast, the linear/linear *function*, which we discuss in Equation 11 in Section 7 is probably relevant solely to the delay region of induced phase-shifting. This is because if the stimulus can produce a moderately strong continuous phase delay shift, it can significantly counteract the intrinsic progression of the circadian pacemaker (due to the longer than 24-hr intrinsic period), thus allowing the stimulus yet more time to persist in the peak delay region. In contrast, a stimulus that can produce a

moderately strong continuous phase advance shift would enhance the intrinsic progression, moving phase away from the peak advance region.

We shall elaborate in Section 4 on the richness of the linear/log function, but first we must comment on the paucity of data from which this function was extracted. The entire data set contains only three phase shift values found for light durations from $T = 15$ seconds (0.25 minutes) to $T = 720$ seconds (12 minutes) (a range of 1.7 log units) and three from $T = 150$ to 390 minutes. The parameter $T_0 = 0.65$ seconds (calculated above) is 1.4 log units shorter than the shortest stimulus duration for which we have data. To support our finding of the linear/log function, therefore, we digress in Section 3 to examine stimulus response in gerbils.

Light pulse duration studies using c-fos induction in the gerbil suprachiasmatic nucleus (SCN)

In casting about for other reports against which to test our proposed linear/log representation of overt circadian response to brief light pulses (e.g., $T < 1,000$ seconds), we discovered the remarkable study by Dkhissi-Benyahya and colleagues (Dkhissi-Benyahya *et al.*, 2000) that uses c-fos induction to evaluate circadian phase shifts to light in gerbils. In Dkhissi-Benyahya *et al.*, wild-caught gerbils (a diurnal species) previously acclimated to a 12:12 light:dark cycle, were placed in constant darkness on test day and stimulated with 500-nm monochromatic light pulses ($3.8 \times 10^{-1} \mu\text{W}/\text{cm}^2$) at CT16 (i.e., 16 hours after normal “lights on” and 4 hours into the “subjective night”); this light stimulus is expected to produce a phase delay. A range of stimulus durations from $T = 3$ seconds to $T = 2,850$ seconds were tested ($n = 4$ per stimulus). Following the light stimulus, animals were returned to total darkness. One hour after the start of light exposure, gerbils were sacrificed, and optical density of c-fos-labeled cells in SCN sections was determined relative to background.

This experiment had at least two clear objectives. The first was to quantify, in terms of optical density of c-fos-labeled cells, the saturation of response with increasing light intensity (in units of photons/cm²/seconds). Their Figure 1A, in which they report 3.74×10^{12} photons/cm²/seconds as the half-saturation intensity for a stimulus duration of 15 minutes, shows success with this objective. We compare this to the 1.4×10^{11} photons/cm²/seconds as the half-saturation intensity for a stimulus duration of 5 minutes in the most extensive saturation study completed by Nelson and Takahashi in the golden hamster (Nelson and Takahashi, 1991); we discuss this study further in Section 5. The similarity of the two results and the underlying physiological relationship supports our hypothesis that optical density of c-fos induction may be comparable to phase shifts in our investigation of the linear/log function; this relationship is also documented in (Trávníčková *et al.*, 1996; Muscat and Morin, 2005).

A second objective of their experiment was to demonstrate that optical density of c-fos induction saturates with increasing stimulus duration from 3 to 2,850 seconds (their Figure 1B). To convert their optical density data to values that we might compare to human phase-shift data, we measured numerical values for these densities (D) from a photo enlargement

of their Figure 1B, then subtracted the reference density (dark control, D_0). The values on the x-axis have been converted to $\log T$ in our Table 2 and Figure 2A.

All 8 points of the gerbil data are plotted in Figure 2A. The first 6 points span the T-range of the data from Nelson and Takahashi (Nelson and Takahashi, 1991), which we discuss in further detail in Section 5. In Figure 2A, we also show the linear/log fit to these first 6 points as a solid line with extrapolation to the two points at longer T as a dashed line. The parameters of this linear/log fit are: intercept $T_0 = 0.69$ seconds, slope $m = 8.76 = \frac{dy}{d \log T}$,

adjusted $R^2 = 0.91$. We compare these data with the human data, for which the linear/log parameters are (from Section 2): $T_0 = 0.65$ seconds, $m = \frac{dy}{d \log T} = 23.13$, adjusted $R^2 = 0.91$.

First, we regard the close match of the T_0 values for human and gerbil (0.65 vs 0.69, respectively) to be remarkable. We propose to rescale the gerbil data by the multiplier $\frac{m_{human}}{m_{gerbil}} = \frac{23.13}{8.76} = 2.64$, and then divide this product by a factor of 1000, to bring the c-fos induction data to the same scale as human phase shift data. These rescaled values are included in Table 2.

We next transfer the log/linear and linear/linear functions for humans from Figure 1B to Figure 2B, including the marker for T^* . To this figure, we add the 8 scaled data points from gerbil. The slope of the first 6 gerbil data points (i.e., in the short T domain) match the slope of the human data exactly, which is expected given our choice of scaling factor. From Figure 2, we observe that the final T in the gerbil data lies beyond T^* , which represents the transition point to the linear/linear process in the human data. It is remarkable how close the data point at the longest T in the gerbil data comes to the human long-T function. The data point at the second longest T in the gerbil data ($T = 900$ seconds) is perplexing, however. As shown in Figure 2B, not only does this data point lie 15 y-units below the human linear/log fit, it also lies approximately 5 y-units below the data at $T = 285$ seconds, which represents a difference in stimulus duration of only 10.25 minutes (615 seconds).

In summary, the central four points of the gerbil data (from $T = 15$ to $T = 285$ seconds) fit our proposed linear/log process extremely well, and the extension to the shortest two data points ($T = 3$ and 9 seconds) is also satisfactory. The data at $T = 2,850$ seconds, which was the longest possible stimulus duration within the experimental framework, suggests a transition out of the linear/log process, not unlike that observed in the human data.

Implications of the linear/log function

We have now presented two mammalian species for which a response measure (phase delay shifts for humans and generation of c-fos-labeled cells in the SCN for gerbils) shows an apparent linear/log process (an increase in phase shift proportional to the logarithm of the duration of light stimulus) of photic stimulation for relatively short stimulus durations. The mathematics of the linear/log function were discussed in Section 2 in Equation 1 and in the two equivalent forms introduced in Equations 1a and 1b. To understand the process that occurs at short duration stimuli, we utilize the concept of yield, $\frac{dy}{dT}$: the ratio of incremental output to incremental “drive”, i.e., the incremental phase-shift per incremental light stimulus

duration. In Equation 1c, we noted that $\text{yield} = \frac{dy}{dT} = \frac{\tilde{m}}{T}$. The interesting features of this result are, first, that yield is not simply declining but being driven down inversely with increasing T and, second, that the relevant time is measured from light onset ($T = 0$) rather than the response onset, T_0 .

In their introduction, Dkhissi-Benyahya and colleagues state: “A single short pulse of light (<1 sec) fails to induce a phase shift of locomotor activity, which generally requires longer durations of light exposure” (Dkhissi-Benyahya *et al.*, 2000). No reference to this statement is given, but by projecting the linear/log function back to T_0 , which is < 1 second in both our human phase delay data and their gerbil c-fos induction data (see Sections 1 and 2), we can quantify the limit hinted at in their statement. At the same time, this backward projection reveals a remarkable finding: yield attains its maximum value, $\frac{\tilde{m}}{T_0}$ at T_0 since T_0 is the shortest possible response onset time. Therefore, T_0 has two possible interpretations: (i) the stimulus duration at which response can first be detected; and/or (ii) the shortest single stimulus duration for which the response can be sustained.

We note that in Equation 1, y could increase without limit (i.e., never saturate). Human phase delay shift data show, however, that this linear/log relationship morphs into a linear/linear relationship (Figure 1A) beginning at approximately $T = 1000$ seconds ($\log T = 3.00$ log seconds) and is complete by approximately $T = 7000$ seconds ($\log T = 3.85$ log seconds). The process that ultimately limits the circadian phase delay shift is complicated and we approach it in Section 7.

A trove of hamster responses to brief light pulses: separating brightness and duration

Throughout our analysis of the effect of stimulus duration so far, the character of the stimulus (e.g., the intensity or the spectrum) was held constant. The coefficient, $\tilde{m}$, however, would also be expected to be an increasing (and probably saturating) function of brightness.

We now turn to the remarkable and comprehensive paper of Nelson and Takahashi (Nelson and Takahashi, 1991) that includes both advance and delay shifts in the nocturnal golden hamster over a stimulus duration (T) range of several log units as well as an intensity (I) range of several log units. From this plethora of riches, we have selected the results shown in their Figures 4 and 5, which are in the phase advance region of the phase response curve. The timing of these pulses (CT19) is in a regime in which response is large but the decline of response rate with time is also strong (e.g., see the PRC in their Figure 2). The data range that is useful to us for the purposes of understanding the short T regime is $T = 300$ seconds (5 minutes). Intensity, I , is reported in terms of photon flux density (photons per cm^2 per second). We use labels of E11 – E14 to designate 10^{11} – 10^{14} photons per cm^2 per second. Light pulses in this study were generated from a tungsten-halogen lamp ($\lambda_{\text{max}} = 503$ nm).

Nelson and Takahashi arranged the experiment by selecting duration, T , on a coarse grid and varying light intensity, I , in small steps. The durations were arranged in a decimal series ($T = 3, 30, \text{ and } 300$ seconds). Raw phase-shift data (phase advances, in minutes) for $T = 300$

seconds were extensively sampled (i.e., multiple animals at more than 12 intensity values) whereas at $T = 30$ seconds only four intensity values were sampled. Similarly, at $T = 3$ seconds, only six intensity values were sampled and only at four intensity values were multiple animals studied. In our Figure 3, we show within these ranges (E11-E14).

Nelson and Takahashi fit a 4-parameter Naka-Rushton function to each T :

$$y = \frac{y_{\max} - y_{\min}}{1 + \frac{\sigma^n}{I}} + y_{\min} \quad (2)$$

where y is the phase shift response in minutes, σ is the stimulus required to achieve the half-maximal response, and n is the steepness or slope of the function. $y_{\max}$ represents the asymptotic fit as $I \rightarrow \infty$ for and $y_{\min}$ represents the asymptotic fit as $I \rightarrow 0$. For $T = 300$ seconds, the raw data are plentiful, and the asymptotes were first fixed by the data (see their Figure 4 caption for details). For $I \rightarrow 0$, the calculated asymptotic shift was -6 minutes, which Nelson and Takahashi indicated was “not significantly different from zero”, whereas for $I \rightarrow \infty$, the calculated asymptotic shift was 114 minutes, which was then used as the maximum possible phase shift for stimulus durations 3, 30, and 300 sec. Note that: (i) the alteration of y_0 from -6 minutes to 0 minutes for $T = 300$ seconds gives us a criterion of ± 6 minutes from which we can assess the relative range for subsequent data manipulation and (ii) contrary to their assumption, a constant maximum shift of $y = 114$ minutes that was calculated for $T = 300$ seconds may not be appropriate for $T = 3$ or 30 seconds; they do not have data at higher intensities for these durations to confirm whether a similar constant maximum shift is achieved.

The Naka-Rushton functions allow calculating the shift, y , for any I and any T ; we chose their four intensities (I) (E11, E12, E13, and E14) and 3 durations (T) (Table 3). We switch the emphasis from the original study (i.e., effect of different intensities at each duration) to the effects of different durations at each intensity. Therefore, our first use was to test whether for intensity I , the linear/log dependence for short T described in Section 2, would also be observed in hamster phase-advance shifts. We conducted an unconstrained linear fit of phase shift, y , vs. $\log T$ to determine the slope, $m(I)$, and intercept $T_0(I)$ for each intensity (I) from Table 3, with results as reported in Table 4. Overall, for the full range of I , the average root mean square deviation (RMSD) was 4.04 minutes (which is within the 6 minute range); we return to this RMSD value below.

Each linear/log fit is characterized by the time parameter, T_0 . Over the selected I range of 1000:1, T_0 ranges from 0.49 to 1.34 seconds, which leads us to an important conjecture: perhaps there is an underlying $\hat{T}_0$ independent of I such that the observed range in T_0 arises solely from noise in the data. This conjecture set us on a search for a suitable constraining $\hat{T}_0$. We realized that imposing any such constraint on T_0 across the 1000:1 range of I must necessarily increase the RMSD; therefore, our aim was to find a $\hat{T}_0$ that minimizes RMSD from the data and maintains an overall RMSD of 6 minutes or less. We therefore conducted a single linear regression using the data across all intensities (rather than individual fits to

each light intensity), which resulted in an overall $\hat{T}_0 = 0.79$ seconds (Figure 3) and an overall RMSD value of 4.85 minutes, which is within our imposed 6-minute range.

With the sole parameter of the linear/log function, T_0 , constrained to a single $\hat{T}_0$ over the entire intensity range, $m(I)$, the influence that light intensity I has on phase shift is contained within the multiplying coefficient, $m(I)$ (Table 4, **constrained fits**). Using this, we discover that the values of $m(I)$ follow a simple logistic function (Figure 5). For comparison, Figure 5 also includes the original $m(I)$ values for the unconstrained linear fits as reported in Table 4. Imposing a constraining T_0 cleans up the presentation of $m(I)$, revealing the innate character of the primary data.

We now rewrite Equation 1, as originally introduced in Section 2, in a more powerful way:

$$y(I, T) = m(I)(\log T - \log \hat{T}_0) \quad (3)$$

Equation 3 suggests that the dependence of shift on stimulus intensity, $m(I)$, and stimulus duration T_0 can be factored into two independent functions, which is known as product separability. We have already discussed in Section 4 that the duration (time) function embodied in Equation 1b (which is linear/log) shows no saturation. In contrast, Figure 5 indicates that the intensity function $m(I)$ does indeed saturate for (intensity) $I \rightarrow \infty$ when $m(I) = 47.5$ minutes, supporting our claim in Equation 3 that duration (time) and intensity factors are independent influences on yield.

We are now able to compare our model predictions (Equation 3, using the constrained fit values of $m(I)$ and $\hat{T}_0$ as reported in Table 4) to the raw data from Nelson and Takahashi that we have reproduced in our Figure 3. Our model predictions (Equation 3) reveal that the asymptotic shift-values for $T = 3, 30$, and 300 seconds are 27.4, 74.9, and 122.4 minutes, respectively. Note that while our product-separable representation fits the Nelson and Takahashi data quite well for $T = 3$ and 300 seconds, the data for $T = 30$ seconds consistently lie below model predictions. The original Naka-Rushton fit to $T = 30$ seconds in Figure 5 of Nelson and Takahashi (Nelson and Takahashi, 1991) is better than our fit, but it appears in that paper that the upper asymptote has been constrained to equal the upper asymptote found for $T = 3,600$ seconds (discussed above), whereas we do not constrain the upper asymptote in our fit for $T = 30$ seconds.

In summary using this data set, we were able to demonstrate:

- A linear/log relationship can represent the relationship between response (phase advance shift, y) and duration, T , for each intensity, I , individually.
- The sole time parameters, T_0 , of the individual linear/log processes could be constrained to a single value, $\hat{T}_0$ that is independent of stimulus intensity, I .
- The response, y , can be represented as the product of an intensity function, $m(I)$, and a duration function, $\log T - \log \hat{T}_0$.

Short and long stimulus duration phase shifting in mouse

For a long time, we despaired of finding studies of non-human phase shifting by light that could be compared to our human short-duration and long-duration stimuli data. Then, we discovered the data of Comas *et al.* (Comas *et al.*, 2006). Because Comas *et al.* used no stimulus shorter than $T = 60$ minutes, we additionally sought a data source that could confirm and quantify the linear/log (short T) behavior for the mouse and found one in the 2007 report by Dkhissi-Benyahya *et al.* (Dkhissi-Benyahya *et al.*, 2007); a report by the same group (Dollet *et al.*, 2010) mostly duplicates these data points and is not included here. These short T data are sparse, as they were intended to provide a baseline validation of their experimental protocol, which primarily addressed phase shifting in mice lacking mid-wavelength cones. The stimulus durations tested were 1, 5, and 15-minute light exposures (480-nm monochromatic light at 2.8×10^{14} photons/cm²/s). We scaled the delay shifts from their Figure 5 to generate our Table 5. These points generate an excellent linear/log fit with $T_0 = 0.79$ seconds and $m = 28.6$ minutes with an adjusted $R^2 = 0.99$, as observed in Figure 6B. We compare these parameters to our human data, where $T_0 = 0.65$ seconds (= 0.011 minutes) and $m = 23.13$ minutes.

Now we are prepared to address the torrent of data in Comas *et al.* (2006). As shown in their Figure 1, they selected 7 stimulus durations of 1, 3, 4, 6, 9, 12, and 18 hours. Light pulses were generated by white fluorescent tubes and achieved an intensity of ~100 lux (~145 mW/m² at the cage floor level). We report in our Table 6 the maximum delay shifts (in minutes) for the relevant T durations (also in minutes) as taken from their Table 1 (Comas *et al.*, 2006). We have added the values for $\log T$ (in seconds) for use in our Figure 6B. We treated these data in the same way we treated the human long duration stimuli data in Section 2: we ignored the data at $T = 60$ minutes (considering it to be transitional, as we discuss further in Section 7) and fitted a linear regression to the remaining 4 points, obtaining $y_0 = 105.6$ minute and $M = \frac{dy}{dT} = 0.38$ with an adjusted $R^2 = 0.94$. This fit is shown in Figure 6A, which is comparable to Figure 1A for the human data. We recall that a similar regression fit to the human data yielded $y_0 = 76.8$ minute and $M = \frac{dy}{dT} = 0.24$ with an adjusted $R^2 = 0.95$.

At this point, we audaciously try to predict short stimulus duration data for Comas *et al.*, if they had undertaken such studies using the same handling and lighting procedures as they did for their long stimulus duration data. The key to such prediction is y_0 , which is a window into short T from long T . The short T parameter we seek to predict is $m = \frac{dy}{d \log T}$.

The data we have in hand that transcends the short- T to long- T transition is the human phase delay shift data. We propose that

$$m_{\text{Comas mouse}} = m_{\text{human}} \frac{y_0, \text{Comas mouse}}{y_0, \text{human}}$$

From the values for human and mouse that we have derived in Section 2 and in this section, we obtain

$$m_{\text{Comas mouse}} = 23.13 \frac{105.6}{76.8} = 31.8 \text{ minutes}$$

For $T_{0,\text{Comas mouse}}$, we would expect $T_0 = 0.78$ seconds as derived from the Dkhissi-Benyahya *et al.* (2007) mouse data to apply directly. We have added to Figure 6B this short-T estimate for the Comas *et al.* data as a dashed line, and note that the predicted slope $m = 31.8$ is close to the slope $m = 28.4$ observed in the Dkhissi-Benyahya *et al.* (2007) data.

We are now in a position to estimate the characteristic stimulus duration, T^* , for the transition in mouse from the linear/log function to the linear/linear function. Using the predicted short-T slope $m = 31.8$ minutes and the observed long-T slope $M = 0.38$ from the Comas *et al.* (2006) data, we find that:

$$T^* = \frac{\tilde{m}}{M} = \frac{m/2.303}{M} = \frac{13.81}{0.38} = 36.6 \text{ minutes}$$

which is remarkably close to $T^* = 37.2$ minutes predicted for humans.

The leaky integrator model: A bridge between extremes of responses

The data that we have just analyzed in multiple species appear to have a discontinuity: for short light pulses (i.e., on the order of a few minutes), there is the linear/log process with a declining yield while for long pulses, the resetting response is a linearly increasing function of T , but with a small yield. An essential feature of the Figure 1B presentation is that the two curves do not intersect. Although prior discussions of the data in Figure 1B have modeled the dynamics using a single four-parameter logistic function (Chang *et al.*, 2012; Rahman *et al.*, 2017), in the leaky integrator analysis formulated below, we propose to embrace these disparate behaviors as limiting cases of a single first-order dynamical system defined by a time constant, B^{-1} , as an alternative model. Before we can develop the leaky integrator model, however, we need to address a fundamental question that we deferred to this section: in defining the long-T linear incremental function, may we use all 4 data points, $T = 60$ minutes, or should we restrict the range to the 3 data points $T > 60$ minutes (corresponding to $T = 150$ minutes in our data set)?

- In Case 1 (3 data points, $T = 150$ minutes), y_0 (intercept) = 76.8 minutes, adjusted $R^2 = 0.95$, M (slope or yield) = 0.27 minutes of phase shift per minute of light exposure.
- In Case 2 (4 data points, $T = 60$ minutes), y_0 (intercept) = 84.4 minutes, adjusted $R^2 = 0.96$, M (slope or yield) = 0.24 minutes of phase shift per minute of light exposure.

Thus, although the addition of the data point $T = 60$ minutes changes both the intercept and yield, we cannot choose one over the other on statistical grounds. A decision can be made only when the linear/log curve for *short* T is considered.

In Section 2, we described the linear/log function in Equation 1b as $y = m(I) \log \frac{T}{T_0}$. The fit to the three short T data points (T = 15, 120 and 720 seconds) yields $m = 23.13$ minutes and $T_0 = 0.65$ seconds with an adjusted $R^2 = 0.82$. We can now convert Equation 1a to the natural logarithm:

$$y = \tilde{m}(I)(\ln T - \ln T_0) \quad (1d)$$

where $\tilde{m}(I) = \frac{m(I)}{2.303} = 10.04$ minutes (where $\ln 10 = 2.303$ when we convert from log to ln).

Now, as Figure 1B shows and as noted above, the short T and long T processes do not intersect, but they come close. Earlier, we undertook to quantify the stimulus duration for which they are closest. This is the value of T for which the functions have matching time derivations. For Equation 1d, as we previously found in Section 2, the matching time derivation is:

$$\frac{dy}{dT} = \frac{\tilde{m}(I)}{T} \quad (4)$$

where both y and $\tilde{m}(I)$ have dimensions in minutes. We chose the dimension for T to be minutes so that $\frac{dy}{dT}$ matches M (i.e., for long T) dimensionally. We then designated the closest approach for the two functions as T^* in minutes. Now, for Case 1 (3 points), we use values from Section 2:

$$M = \frac{\tilde{m}(I)}{T^*} \text{ or } T^* = \frac{\tilde{m}(I)}{M} = \frac{10.04}{0.27} = 37.19 \text{ minutes}$$

For Case 2 (4 points), we use values from this section (Section 6):

$$T^* = \frac{\tilde{m}(I)}{M} = \frac{10.04}{0.24} = 41.83 \text{ minutes}$$

The message here is that this parameter T^* , which characterizes the transition process, is surprisingly close to 60 minutes, which places that data point in the transition zone. Therefore, Case 2 (with one point at ~60 minutes) was rejected from the long T fit.

It is with these findings in hand, therefore, that we elected in Figure 1B to show the Case 1 (3 points) representation for the long T function, and added a marker for $T^* = 37.19$ minutes ($T^* = 2,260$ seconds, $x = \log T = 3.35$). In the leaky integrator analysis below, we suggest that T^* is equivalent to the leaky integrator time constant.

We now focus on this transition between behaviors for short T and long T.

Origins

There are some key points to suggest a “leaky integrator” type physiology:

- For short T, there is a linear/log response, for which yield (phase shift per unit of stimulus duration) is initially very high (139.2 minutes of phase shift for each

minute of light exposure in humans, as noted in Section 2) and then declines inversely as the stimulus duration (T) increases (see inset of Figure 1A). This decline is continuous and approaches zero.

- For long T (T > 60 minutes), however, the yield does not asymptote to 0, but instead maintains a steady non-zero yield with increasing light exposure duration [about 0.27 minutes (~16 seconds) of phase shift per minute of light exposure in humans]. Though the yield resulting from long stimulus durations is much lower than for short stimulus durations, when the stimulus duration is long, the sum of the resulting incremental phase shifts can be substantial.
- Between the “inverse T” linear/log relationship of short duration light pulses to the “steady yield” linear/linear relationship of long duration stimuli, there appears to exist a transition.

The transition to a steady yield implies that the integrator process is imperfect. For a perfect integrator, yield would continue to decrease over time as the duration of the stimulus (T) increases. Our results show that the integrator reaches a steady state at some finite value (i.e., the yield fails to decline with the inverse of T as T increases). Therefore, we chose to model the process as a “leaky” integrator. The example of a leaky integrator taught in mathematics classes is a reservoir with in-flowing water. If the reservoir is a perfect integrator, the total (i.e., integrated) amount of water in it will continue to rise indefinitely. If the reservoir leaks, then the total amount of water may (in some circumstances) reach a fixed level. The analogy to phase resetting would be the sum of the photons as measured by stimulus duration (T) (instead of in-flowing water) being inversely related to the rate of the change of the phase shift (instead of amount of water). Note that a strict photon counter-type physiologic response cannot produce this observed behavior. The yield is inversely related to the rise of the total amount of water in the reservoir. For a perfect integrator, the yield will decrease because the total amount of water in the reservoir increases. For a leaky integrator, the amount of the leak is related to the amount of water in the reservoir, and therefore the yield will reach a steady state (which may be zero if it reaches a fixed level) as water flows both in and out.

The imperfect “leaky” integrator

To keep the mathematics simple, we envision that the process begins at the onset of integration, T = 0. Let R be the output of the (defective) integrator and consider:

$$\frac{dR}{dT} = 1 - BR \quad (5)$$

If B = 0, then $\frac{dR}{dT} = 1$ and R is simply T itself, i.e., a perfect integrator. A positive value for B implies outflow from the reservoir, R (i.e., a “leak”). As R increases, so does outflow. Finally, a balance ($\frac{dR}{dT} = 0$) is reached when BR = 1: outflow matches inflow. The entire transient process is described by a particular integral of Equation 5:

$$R = B^{-1}(1 - e^{-BT}) \quad (6)$$

Note that at $T = 0$, $R = 0$ and that $R = B^{-1}$ is the asymptotic value for R as T increases. Both R and B^{-1} are time parameters, and B is therefore a rate parameter. As we approach the asymptote, the value of B characterizes the rate of approach. For what is to follow, we comment that, parameters aside, the functional dependence of R on T is simply $1 - e^{-BT}$.

In describing the linear/log process, we wrote the yield function in Equation 4 as $\frac{dy}{dT} = \frac{\tilde{m}(I)}{T}$, where $\tilde{m}(I) = \frac{m(I)}{2.303}$ and T is the perfect integrator. For the leaky integrator, we replace T by $R(T)$:

$$\frac{1}{\tilde{m}(I)} \frac{dy}{dT} = \frac{1}{R(T)} = \frac{B}{1 - e^{-BT}} \quad (7)$$

The open integral of Equation 7 is:

$$\frac{y}{\tilde{m}(I)} = \ln(e^{BT} - 1) \quad (8)$$

We observe that for $BT \gg 1$, $\frac{y}{\tilde{m}(I)} \rightarrow BT$, which is the linear/linear (long T) process.

We understand that the linear/log process (short T) originates at T_0 . Consequently, we convert Equation 8 to a definite integral originating at T_0 :

$$\frac{y}{\tilde{m}(I)} = \ln(e^{BT} - 1) - \ln(e^{BT_0} - 1) \quad (8a)$$

Equation 8a represents the complete leaky integrator solution, and introduces a single parameter, the rate constant B . It is easier to interpret this model in terms of the time constant, B^{-1} , which we will now do.

Limiting cases

Note that B^{-1} establishes a timescale for the transition between the linear-log process (short T) and the linear-linear process (long T). We look at Equation 11 for these two limiting cases. In the context of human data, B^{-1} lies somewhere between $T = 12$ and $T = 60$ minutes (Figure 1).

Limiting Case 1, at short T , where $T \ll B^{-1}$ and $T_0 \ll B^{-1}$:

In this case, $(e^{BT} - 1) \rightarrow BT$ and Equation 8a becomes

$$\frac{y}{\tilde{m}(I)} = \ln(BT) - \ln(BT_0) \quad (9a)$$

$$= \ln(T) - \ln(T_0) = \ln\left(\frac{T}{T_0}\right) \quad (9b)$$

which is simply the linear-log relationship as in Equation 1c.

Limiting Case 2, at long T, where $T \gg B^{-1}$ or $BT \gg 1$:

In this case, $\ln(e^{BT} - 1) \rightarrow BT$. Since $BT_0 \ll 1$, however,

$\ln(e^{BT_0} - 1)$, remains $\ln(BT_0)$ as in the short T case.

Now Equation 8a becomes:

$$\frac{y}{\tilde{m}(I)} = BT + \ln\left(\frac{1}{BT_0}\right) \quad (10)$$

or

$$y = (\tilde{m}(I)B)T - \tilde{m}(I)\ln(BT_0) \quad (10a)$$

which we rewrite simply as

$$y = AT + y_0 \quad (11)$$

the familiar linear/linear function.

Evaluating the “leak” time constant

Since A (the slope in Equation 11) is now seen simply as $\tilde{m}(I)B$, we can evaluate

B^{-1} as:

$$\frac{1}{B} = \frac{\tilde{m}(I)}{M} = \frac{10.04}{0.27} = 37.19 \quad (11a)$$

using values from Section 2. This is exactly the stimulus duration, T^* , found in Section 2 for which the linear/log and linear/linear functions had their closest approach (i.e., matching time derivatives).

We must note that the paired Equations 10 and 11 also embody a second equivalence:

$$y_0 = -\tilde{m}(I)\ln(BT_0) = \tilde{m}(I)\ln\left(\frac{B^{-1}}{T_0}\right) \quad (11b)$$

which may be solved for B^{-1} as follows:

$$B^{-1} = T_0 e^{\frac{y_0}{\tilde{m}(I)}} \quad (11c)$$

Just as Equation 11a provides an estimate of B^{-1} from the time derivatives embodied in the asymptotic functions, Equation 11b incorporates the data provided by the intercept y_0 . We now examine Equation 11b further.

In Equation 11b, the units of B^{-1} and T_0 must match. We choose units of minutes, in which case T_0 becomes $T_0 = 0.011$ minutes (= 0.65 seconds). Since we already have a solid estimate of $B^{-1} = 37.19$ minutes from Equation 11a, we use this along with T_0 and $\tilde{m}(I) = 10.04$ minutes to calculate an estimate of y_0 (i.e., the phase shift at T_0) via Equation 11b. We find this to be $y_0 = 81.5$ minutes. The original linear regression to the three longest duration stimuli described in Section 2 gave $y_0 = 76.8$ minutes, which is 7% less than this estimate. If y_0 is 76.8 minutes (from Section 2 rather than from Equation 11a,b), then B^{-1} is 22.7 minutes (Equation 11c), which is a 40% reduction over the Equation 11a estimate. Thus, as expected from a log or exponential (not linear) function, a 7% reduction in y_0 results in a 40% reduction of B^{-1} .

We understood when y_0 was first recognized in Section 2 that it was an embodiment of all the high response sensitivity to “early” photons. Equation 11b contains the two parameters T_0 and $\tilde{m}(I)$ that characterize the “early” (short T) linear-log process. That Equation 11b predicts y_0 to within 7% is quite remarkable. Equation 11c, however, proves to be a very inaccurate tool for evaluating B^{-1} . We must rely solely on Equation 11a for evaluating B^{-1} .

Discussion

Summary

We have undertaken to describe the responsiveness of the circadian pacemaker in several mammalian species to data that include the wide range of stimulus duration from 15 seconds to 6.5 hours within a single experimental protocol. First, in the first few minutes of the light exposure, the circadian system is maximally responsive. Second, the system continues to be responsive to longer duration light stimuli, though at a reduced phase-resetting yield. Third, these properties are common across several mammalian species. Finally, the impact of intensity and duration on the resetting response appear to be functionally separable; that is to say, the temporal dynamics of the phase resetting response to light stimulus duration are independent of intensity. These findings, if confirmed by further experiments, have important practical implications in terms of the design of lighting regimens to reset the mammalian circadian pacemaker.

Let us once again summarize the assumptions embedded within the linear/log concept. First, the entire duration dependence of shift response within the short-T linear/log regime (be it delay or advance shift) appears to be well described by a single parameter, T_0 . We find the value of T_0 of ~0.7 seconds to be remarkably similar across mammalian species. Second, extrapolation of data downward to T_0 suggests that no overt shift response will be observed for a single light stimulus when $T < T_0$. Third, yield (added shift per element of added stimulus duration) is very high for T of approximately 1 second, but decreases dramatically as T is extended. Yield does not decline gracefully (exponentially) in this regime but is actually driven downward inversely with T. Finally, based on the remarkable data from Nelson and Takahashi (Nelson and Takahashi, 1991), spanning both 3 log units of stimulus

duration T and 4 log units of intensity I , we suggest that shift-response can be factored into the product of distinct functions for duration T and intensity I , where the duration function is linear/log and the intensity function follows a traditional Naka-Rushton relationship.

Our findings on the long- T linear/linear response to stimuli in excess of 1 hour, as observed in both human and mouse data, is somewhat protocol-dependent. First, this linear/linear response depends on operating within the phase-delay region of the traditional PRC. Second, the light stimulus should be sufficiently strong such that the natural progression of the circadian pacemaker can be reversed (“shifted”) by the light stimulus as occurs during a phase delay. The linear/linear relationship also requires that such reversal be achieved promptly, as the “prompt” data of Dkhissi-Benyahya *et al.* imply (Dkhissi-Benyahya *et al.*, 2000). Consider, for example, the consequence of a prompt realization of a 45-minute phase delay (which we have measured on a subsequent circadian cycle) to a 2-minute light pulse (Rahman *et al.*, 2017). At the end of the light pulse, the pacemaker has been driven backward 45 minutes. In contrast, at the end of our 390-minute light exposure, the pacemaker has been driven backward by only 183 minutes. These responses act to keep the pacemaker at, or near, the peak delay response of the PRC. In fact, the mouse data we take from Comas *et al.* (Comas *et al.*, 2006) are from a column they correctly labeled “maximum delay”, indicating that these responses are indeed near the peak sensitive region of the PRC.

The wonderful phase shift data resource of Nelson and Takahashi has led to several remarkable interpretations. First, we found evidence for a saturating logistic function of intensity, $m(I)$, that supplies a multiplicative rate parameter to the temporal function (Figure 5). Second, we found evidence for a linear/log temporal function of duration T that appears to be universal for all intensities. For reasons that we laid out early in our analysis of the Nelson and Takahashi data, these principles extend to durations of 5 minutes and perhaps several minutes beyond, but certainly not to 60 minutes. The linear/log function is characterized by a “start time” of $T_0 = 0.79$ seconds independent of light intensity over the range of intensities available. By imposing a universal T_0 constraint on the entire Nelson and Takahashi data set (Section 5), the overall response function $y(I, T)$ is decomposed into a simple product of an intensity function, $m(I)$, and a temporal function, $(\log T - \log T_0)$. The intensity function saturates for large I (see Figure 5). The temporal function shows the incremental response decreases inexorably for large T , but the accumulated response does not saturate over the range of durations studied.

The linear/log temporal function that we extracted from the Nelson and Takahashi data has proven extraordinarily useful. Both the gerbil data from Dkhissi-Benyahya *et al.* (Dkhissi-Benyahya *et al.*, 2000) (for 3 seconds $\leq T \leq 285$ seconds) and our human data (Chang *et al.*, 2012; Rahman *et al.*, 2017) (for 15 seconds $\leq T \leq 720$ seconds) were well fit by this function and gave T_0 values of 0.69 seconds and 0.65 seconds, respectively, which compare well to the universal $T_0 = 0.79$ seconds derived from the Nelson and Takahashi data set.

Our central challenge is understanding human phase delay shift data. We already knew that for $T > 60$ minutes, the phase shift increases linearly with T up to $T = 390$ minutes (i.e., 6.5 hours). When this linear function was projected back to $T = 0$ (giving an impossible phase shift of $y_0 = 76.75$ minutes for a light stimulus of 0 seconds), it was clear that significant

shift had occurred before $T = 60$ minutes. This led us to initiate experiments for shorter duration T (Chang et al., 2012; Rahman et al., 2017). These data revealed that there was a linear/log relationship for $T < 60$ minutes, similar to data that have been observed in other species. Now we had two limiting behaviors: linear/log for $T < 3,600$ seconds (< 1 hour) and linear/linear for $T > 3,600$ (> 1 hour) seconds. The mechanism that we proposed to approximate these limiting behaviors was the leaky integrator. The leaky integrator introduced only one new parameter (other than those already observed in the limiting cases): the rate constant, B , or what is more easily understood, the time constant B^{-1} .

The full leaky integrator solution, which embraces both limit cases, is defined in Equation 8a. We have simplified Equation 8a for the limit cases. The interesting feature is that these limit cases do not intersect; there is always a gap between the linear/log and the linear/linear portions for which the full leaky integrator equation must be used. We also have calculated the exact value for this gap as $T = B^{-1}$ as 37.2 minutes. In humans, we observe that the phase shift estimated by Equation 8a at $T = 37.2$ minutes is ~ 87 minutes, whereas the extrapolation of the linear/log function to $T = 37.2$ minutes yields a phase shift of ~ 82 minutes. The difference is within the error limits, given the relatively small data sets.

In their Figure 2B, Dkhissi-Benyahya *et al.* (Dkhissi-Benyahya *et al.*, 2000) try to fit a saturating Naka-Rushton equation to the c-fos-labeled cell density vs. T . There is a widespread belief that response to increasing T must saturate just as response to increasing I does. The leaky integrator model is based on the concept that incremental response saturates with increasing T , while cumulative response does not until a different part of the phase response curve is reached. The leak-rate, B , is also a purely temporal concept, and so we might expect that it, like T_0 , will prove to be independent of I . As we cited above, the values of T_0 lie in a variation range of 7% across hamster, gerbil, and human. We look forward to learning whether the various B^{-1} will be close to our estimate of 37.2 minutes as more species are studied over a range of T that includes both the linear/log and linear/linear behaviors.

Caveats and Future Work

There is much work to be done to extend and validate the work presented here. Firstly, given the small number of data points and variability in data, no one fit or model can currently be chosen as the most appropriate. Each mathematical fit has different physiological implications; presenting more than one possible physiological basis for the observed results can be used to develop new experiments to better define the physiology, since each will have different predictions under specific conditions. There is a paucity of phase-resetting data in the stimulus duration regime between 12 and 60 minutes for humans, and 15 and 60 minutes for mice. This regime is where we have predicted the transition from linear/log to linear/linear to occur. Specifically, we predict this transition to occur at $T^* = \sim 37.2$ minutes. Future experiments in humans and mice should therefore focus on light-stimulus durations within this regime using the same experimental protocols as in the existing studies to confirm the leaky integrator concept.

We have not considered here the physiological mechanism underlying the leaky integrator concept. We note, however, that the prompt resetting response observed in the short duration

linear/log regime may reflect contribution of the cone photoreceptors in the retina. For example, a prior study from our group (in humans) observed that melatonin was more strongly suppressed during the first 30 minutes of exposure to monochromatic 555 nm light (a stimulus expected to activate primarily the cone photoreceptors) compared to monochromatic 460 nm light (a stimulus expected to activate primarily the melanopsin-expressing intrinsically photosensitive retinal ganglion cells [ipRGCs]), whereas melatonin was more strongly suppressed for stimulus durations longer than 30 minutes by the monochromatic 460 nm light (Gooley et al., 2010). A prompt response to stimulation of the cone photoreceptors and a sluggish but sustained response to stimulation of the melanopsin-expressing ipRGCs has also been observed in the pupillary light reflex (Alpern and Campbell, 1962; Bouma, 1962; Lucas et al., 2003; Gamlin et al., 2007; Mure et al., 2009; Gooley et al., 2012) and phase resetting (Panda et al., 2002; Hattar et al., 2003; Do and Yau, 2010; Gooley et al., 2010). Duration-response experiments that span both the short T and long T regimes are needed in both animals (i.e., rod/cone and melanopsin knockouts) and humans (monochromatic blue versus monochromatic green light exposures and/or in blind individuals with intact ipRGCs) to understand the contributions of the different photoreceptor systems to our leaky integrator concept.

In prior works, we have proposed other mathematical formulations to describe the phase resetting effects of light (Kronauer et al., 1999; Kronauer et al., 2000; St. Hilaire et al., 2007; Chang et al., 2012; Rahman et al., 2017). For example, the light preprocessor (Process L) of our mathematical model of the effects of light on the human circadian pacemaker (e.g., Kronauer et al., 1999; Kronauer et al., 2000; St. Hilaire et al., 2007) was formulated as a non-linear process in which the first 5–10 minutes of a light stimulus elicited a rapid increase in phase resetting response followed by a slower increase in the rate of phase resetting after a critical light stimulus duration was released. The principles of the leaky integrator model introduced here are similar to this Process L formulation. Indeed, the distinctive shape of the leaky integrator model—logarithmic for short T, linear for long T—is similar to what is observed with integration of $(1-n(t))$ from 0 to T, where $n(t)$ is taken from Process L. However, the leaky integrator model incorporates new properties that have emerged from the available data since Process L was proposed, including (1) strong phase resetting in response to short stimulus durations (e.g., T = 15 seconds and 2 minutes) in humans; (2) identification of the critical duration window within which the response transitions from a rapid increase in phase resetting to a slower rate of increase (i.e., between T = 12 and 60 minutes); and (3) discovery of the minimal stimulus duration T_0 at ~0.65 seconds. In addition, (i) Process L does not have the property of the two regimes (log/linear and linear/linear) that we have identified, which is an important feature at short T that emerges from the data we have analyzed here. (ii) Process L would need significant reparameterization to generate the phase shifts observed at short T; and this reparameterization then fails to reproduce other properties of the effects of light on the circadian pacemaker (unpublished work). We have also previously used a 4-parameter logistic function to describe the relationship between stimulus duration and phase resetting response (Chang et al., 2012; Rahman et al., 2017). All three of these mathematical formulations provide a reasonable fit to the data, but given the sparsity of the data sets it is not possible to distinguish statistically which one is the best. A comparison of these models

to determine which model best represents the physiology should be done after additional experiments are performed.

Finally, here we have only considered the resetting response to a single light stimulus. In a prior study in humans, we reported that a single sequence of intermittent light (six 15-minute bright light pulses separated by 60 minutes of very dim light; 6.5 hours total) yielded a phase shift that was 73% of the phase shift induced by a 6.5-hour continuous bright light stimulus despite representing only 23% of the continuous stimulus duration (Gronfier et al., 2004). Similar findings have also been reported in mice (Comas *et al.*, 2007). More recently, Zeitzer and colleagues have demonstrated that sequences of 2-millisecond light flashes can elicit significant phase resetting responses (Zeitzer et al., 2011; Najjar and Zeitzer, 2016). These findings suggest that the system is capable of integrating a series of consecutive light stimuli, even if they are very brief (i.e., shorter than our estimated T_0 value). The current version of our leaky integrator model cannot address integration of the light input signal over intermittent exposures; further work is needed to understand this phenomenon. We further note that while our estimated T_0 value of ~0.7 seconds, which represents the minimal stimulus duration at which a response is elicited, is not consistent with the findings of multiple millisecond flashes, it is consistent with the finding from Nelson and Takahashi (1991) that a single 3-millisecond pulse elicits no measurable response; to our knowledge, single millisecond flashes have not been tested in humans.

Conclusion

These findings highlight consistencies in the temporal dynamics of circadian resetting responses to single duration light exposures across species and further challenge the notion of the circadian phase resetting system as a photon counter. We look forward to the results of future experiments and modeling work.

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Figure 1:

(A) The human duration response curve spanning very short (0.25 minutes) to long (390 minutes) light exposure durations reveals a non-linear relationship with approximately linear response for $T > 60$ minutes (omitting $T = 60$ minutes from the fit; circles, solid line) and approximately log response for $T < 60$ minutes (triangles, dashed line). The inset shows the yield (resetting rate, $\frac{y}{T}$) as a function of light exposure duration. (B) The same phase delay shifts plotted on a log scale for $T > 3600$ seconds (i.e., omitting $T = 3600$ seconds from the fit; circles, solid line) and $T < 3600$ seconds (triangles, dashed line). The vertical line indicates the approximate light exposure duration at which a transition between asymptotic states occurs, as described in Section 6.

Figure 2:

(A) Optical density of c-fos induction in the SCN in response to a range of stimulus durations (log T, seconds), as originally reported in Dkhissi-Benyahya *et al.* (2000), was scaled by the optical density in the dark control and divided by a factor of 1000 for ease of display. Data over the range of $T = 3$ to $T = 285$ seconds (filled squares) were fit by a linear regression (solid line) with an extrapolation to $T = 2850$ s (dashed line) for data for $T > 300$ s (open squares). (B) Optical density of c-fos induction in gerbils (square symbols and solid line fit with dashed-line extrapolation to $T = 2850$ s) and linear/log and linear/linear fits (dashed lines) to phase delay shifts in humans (from Figure 1B) are plotted on the same graph for comparison.

Figure 3:

A comparison of the raw golden hamster phase advance data (mean $\pm$ sem) as originally reported in Nelson and Takahashi (filled triangles, $T = 3$ sec; open circles, $T = 30$ sec; filled squares, $T = 300$ s) with estimates of our linear/log model (solid lines, model as reported in Equation 3). The light gray bar denotes the irradiance range over which our analysis was focused.

Figure 4:

Fits to golden hamster phase advance data (minutes) as a function of stimulus duration (T , seconds) transformed into $x = \log T$ (reproduced from Nelson and Takahashi). Constrained linear/log fits (with a universal $\hat{T}_0 = 0.79$ sec).

Figure 5:

Relationship between intensity (or Irradiance, I) and slope $m(I)$ using values from Table 4 (derived from linear fits with universal $\hat{T}_0$). The solid line is the fit by the logistic function in Equation 6; the horizontal dashed line denotes the asymptote of this fit. Slope values derived from the unconstrained fits (Table 4) are included for comparison (open triangles).

Figure 6:

(A) Phase delay shifts in mouse (open circles; from Comas *et al.*, 2006) in response to long duration stimuli. The solid line indicates a linear-linear fit to the long duration data. The data point at $T = 60$ minutes was omitted from the fit, as indicated by the extrapolated dashed line. (B) The phase delay data over long duration stimuli (open circles) are plotted in a linear/log manner against phase delay shifts in mouse in response to short duration stimuli (open triangles; from Dkhissi-Benyahya *et al.*, 2007). The solid line to long duration represents the same fit as plotted in panel A. The dashed line represents the fit of the short duration data, and the solid line fit over this same range represents the fit that would be predicted by the long duration data.

Table 1:

Phase delay shifts and shift rate of the human duration response curve.

T: duration of light exposure (minutes)	Y: delay shift (median $\pm$ SD, minutes)	$\frac{y}{T}$: resetting rate (i.e., minute of phase shift per minute of light exposure)
0.25	34.8 $\pm$ 47.2	139.2
2.00	45.4 $\pm$ 28.4	22.7
12.00	74.2 $\pm$ 35.2	6.2
60.00	102.6 $\pm$ 35.6	1.7
150.00	120.6 $\pm$ 37.2	0.8
240.00	134.5 $\pm$ 46.8	0.6
390.00	183.0 $\pm$ 27.0	0.5

Table 2:

c-fos induction in the SCN in response to a range of stimulus durations (reproduced from Figure 1B of Dkhissi-Benyahya et al., 2000).

T (sec)	$x = \log T$	$y = D - D_0$	Scaled y
3	0.48	7,450	19.67
9	0.95	6,020	15.89
15	1.18	12,400	32.74
28	1.45	14,930	39.42
90	1.95	18,810	49.66
285	2.46	22,910	80.48
900	2.95	21,040	55.55
2,850	3.46	34,030	89.84

Table 3:

Phase advance shifts (seconds) in the golden hamster at different intensities across durations

T, seconds	E11	E12	E13	E14
3	9.9	14.9	21.9	31.3
30	19.3	33.0	51.1	70.5
300	38.4	76.2	101.4	110.6

Table 4:

The results of unconstrained and constrained linear/log fits to golden hamster phase advance data.

	Unconstrained fits				Constrained fits			
I	m	x₀	T₀	RMSD	m	x₀	$\hat{T}_0$	RMSD
E11	14.2	-0.11	0.79	2.26	14.3	-0.10	0.79	2.26
E12	30.7	0.13	1.34	5.94	27.2	-0.10	0.79	6.75
E13	39.7	0.01	1.03	4.98	37.5	-0.10	0.79	5.40
E14	39.6	-0.31	0.49	0.20	43.8	-0.10	0.79	3.78
mean ± SD		-0.07 ± 0.19	0.91 ± 0.36					

Table 5:

Phase-shifting response to short light durations in mice (Dkhissi-Benyahya et al., 2007).

T (sec)	log T (sec)	Shift, y (min)
60	1.78	54.0
300	2.48	73.5
900	2.95	87.5

Table 6:

Phase-shifting response to long light durations in mice (Comas et al., 2006).

Mouse		
T (min)	log T (sec)	Shift, y (min)
60	3.56	125.4
180	4.03	159.0
240	4.16	209.4
360	4.33	248.4
540	4.51	303.6