# Velocity Response Curves Support the Role of Continuous Entrainment in Circadian Clocks

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#### Abstract

Circadian clocks drive endogenous oscillcations in organisms across the tree of life. The Earth's daily light/dark cycle entrains these clocks to the environment. Two major theories of light entrainment have been presented in the literature – the discrete theory and the continuous theory. Here, we re-introduce the concept of a velocity response curve (VRC), which describes how a clock's speed is adjusted by light. We examine entrainment of a mathematical model of the circadian clock using both the VRC and phase response curves (PRCs) for long (circa 12 h) pulses of light. Our results demonstrate that the VRC and PRCs together predict clock behavior under full photoperiod entrainment, supporting the contention that the clock is being adjusted continuously. Further, we show that much of the insights gained from PRCs and the discrete theory of entrainment can be used to understand continuous entrainment. For example, we show that the presence of a deadzone in the VRC explains why photoskeleton and full photoperiod entrainment yield the same phase of entrainment.

*Keywords*: phase response curves, mathematical modeling, circadian clock, sensitivity analysis, entrainment, velocity response curves

# Introduction

In organisms across the tree of life, circadian clocks coordinate daily behaviors with the environment by responding to external cues, or zeitgebers. Left in constant conditions, these clocks will oscillate, but with a period that is not exactly 24 hours. Entrainment allows zeitgebers, such as daily light/dark cycles, temperature cycles, and social interactions to adjust the period and synchronize the clock with the environment. Historically, the circadian field has employed two theories of light entrainment – the discrete theory of Colin Pittendrigh and the continuous theory of Jürgen Aschoff<sup>1</sup>. The discrete theory assumes that the light/dark transitions at dawn and dusk reset or phase shift the clock instantly, correcting its mismatch with the environment. These transitions are mimicked experimentally as short pulses of light. Thus, the phase response curve (PRC), which measures the phase shift resulting from a short pulse of light, is the main experimental tool and has been a good predictor of entrainment behaviors (Daan and Pittendrigh, 1976a). Alternatively, the continuous theory assumes that light acts on the clock throughout the circadian cycle. It is supported by evidence that the period of an animal's clock depends upon the levels of light in constant conditions (Aschoff, 1979). It is now generally accepted that both theories are at least partially valid. Reconciling them remains a perplexing but important question.

Three unified theories of entrainment have been presented. First, Pittendrigh himself (Daan and Pittendrigh, 1976b) considered the possibility of velocity response curves (VRCs) (Swade, 1969), which are similar in shape to PRCs but describe changes to the clock's phase *velocity*. In this case, the majority of the phase shifting would take place at dawn and dusk not because the light/dark transition is important but because the clock's velocity cannot be altered during the middle of the day (see Figure 1A). Second, the "limit cycle" interpretation of Peterson (1980) raises the possibility that there are two separate limit cycles used by the molecular clock – one in constant darkness (DD) and another in constant light (LL). Movement between the two cycles would cause discrete and continuous entrainment to look similar at the behavioral level but would be different at the molecular level (Johnson et al., 2003). Third, Beersma *et al.* (1999) present a phase-only model incorporating continuous effects (using a period response curve) and discrete effects (using a phase response curve), which are treated as independent from one another. This model takes into account variability in light patterns, illustrating that a combination of the two effects yields the most robust entrainment behavior. All three theories were developed prior to the development of mathematical

<sup>&</sup>lt;sup>1</sup>For recent perspectives on entrainment, see (Johnson et al., 2003) and (Roenneberg et al., 2003) and the references contained within.

modeling at the molecular level. Thus they have not been evaluated at the molecular level – a necessity for determining the biological mechanisms for entrainment.

In mammals, the master clock resides in the suprachiasmatic nuclei (SCN) of the hypothalamus where transcriptional feedback networks drive oscillations in thousands of neurons. The SCN receives environmental timing information through the retinohypothalamic tract. The pathway of light information is not well characterized, but there is evidence that short pulses of light cause rapid induction of mRNA transcription from clock genes *Period1 (Per1)* and *Period2 (Per2)* (Reppert and Weaver, 2001). Additional evidence indicates that the effects of light are attenuated over time via either saturation or adaptation (Comas et al., 2006; Comas et al., 2008). Further, for nocturnal mammals, light fails to cause any phase shifts during a clock's internal, or subjective, day. This is evident in the so-called deadzone of their PRCs. It has been argued that a clock-controlled, or phase-dependent, gate prevents light from entering the system during the day (Roenneberg et al., 2003; Geier et al., 2005).

In the present work, we study the process of entrainment using a mathematical model of the mouse circadian clock that incorporates both "saturation" and phase-dependent light gates. We use a limit cycle model and an analytical VRC to demonstrate the relationship between the PRC of the discrete theory and the period-modulation of the continuous theory, thus providing mathematical support for a VRC unified theory of entrainment. We demonstrate the ability of our unified theory to predict properties such as the phase angle of entrainment and show that much of the intuition gained by studying PRCs can be used to understand continuous effects of light. We also show that the presence of a deadzone of the VRC explains why pulse and continuous stimuli at subjective dawn yield the same response and further that the VRC acts as the basis on which PRCs of differing durations are formed.



Figure 1: Velocity response curves (VRCs) in free-run and under entrainment. A) The VRC has a shape similar to that of a PRC, but shows changes in the clock's velocity. When the VRC is one, then light (L = 1) doubles the speed of the clock. The VRC is shown as its changes under B) full photoperiod (12:12) and C) skeleton photoperiod (1:10:1:12) entrainment. D) Shown is the difference between zeitgeber time and the phase of the clock under both entrainment scenarios (solid line for the full photoperiod, dashed line for the skeleton photoperiod). Positive values indicate the internal phase is ahead of zeitgeber time. The dark gray background indicates night in both scenarios. The light gray background from ZT1 to ZT11 indicates the period of darkness during photoskeleton entrainment.

# **Materials and Method**

## **Phase Sensitivity Measures**

## Limit Cycles and Phase

A limit cycle model is a deterministic model whose solution is a stable, attracting cycle, or *limit cycle*  $\gamma$ . It is defined by a set of autonomous nonlinear ordinary differential equations

$$\dot{\mathbf{x}}(t) = \mathbf{f}(\mathbf{x}(t, \mathbf{p}), \mathbf{p}) \tag{1}$$

where  $\mathbf{x} \in \mathbb{R}^n$  is the vector of states and  $\mathbf{p} \in \mathbb{R}^m$  is the vector of (constant) parameters. A simulation of the freerunning clock (i.e. with the nominal DD parameter set) will be along the limit cycle (i.e.  $\mathbf{x}^{\gamma}(t, \mathbf{p})$ ) and will have period  $\tau$  (i.e.  $\mathbf{x}^{\gamma}(t, \mathbf{p}) = \mathbf{x}^{\gamma}(t + \tau, \mathbf{p})$ .

The phase  $\phi$  of a clock model is indicated by *position* on limit cycle, e.g the position of the peak of *Per2* mRNA is associated with CT7 ( $\phi = 7$ )<sup>2</sup>. In constant conditions,  $\phi(\mathbf{x}^{\gamma}(t))$  will progress at the same rate as external, or zeitgeber, time<sup>3</sup>. The single ODE describing its trajectory is

$$\frac{d\phi(\mathbf{x}^{\gamma}(t,\mathbf{p}))}{dt} = 1, \qquad \phi(\mathbf{x}^{\gamma}_{\mathbf{0}}) = 0$$
<sup>(2)</sup>

where  $\mathbf{x}_{\mathbf{0}}^{\gamma}$  is the position on the limit cycle  $\gamma$  associated with dawn (Kuramoto, 1984; Brown et al., 2004)<sup>4</sup>.

When light acts on the clock model, it is manifested as a parametric perturbation. Parametric perturbation causes the state trajectory to leave the DD limit cycle. Thus, our definition of phase must be extended to positions off the limit cycle. For this, we use isochrons. An isochron is a hyperplane that acts as a "sametime locus" (Winfree, 2001); over time, all points on a single isochron approach the same position on the limit cycle, and therefore all share the same phase. In the interest of concise notation, we write  $\phi(\mathbf{x}(t,\mathbf{p}))$  as  $\phi(\mathbf{x}(t))$  below.

#### **Dynamic Phase Tracking**

To predict the phase dynamics in response to a series of infinitesimally short perturbations, we consider two forms of the phase evolution equation and infinitesimal phase response curve. They are rooted in theory established by Winfree and Kuramoto, and extended by others (Kuramoto, 1984; Kramer et al., 1984; Winfree, 2001; Brown et al., 2004; Taylor et al., 2008). The oldest measure, the state impulse PRC (sIPRC)<sup>5</sup>, predicts the phase response to a direct manipulation of a state trajectory. The sIPRC is a vector, with one entry sIPRC<sub>k</sub> for each state  $x_k$ :

$$\mathrm{sIPRC}_k(\phi(\mathbf{x}^{\gamma}(t))) = \frac{\partial \phi}{\partial \mathbf{x}_k^{\gamma}}(\phi(\mathbf{x}^{\gamma}(t))),$$

 $<sup>^{2}</sup>$ CT7 is circadian time 7, that is 7 circadian hours after subjective dawn. In keeping with standard practice, we associate CT0 with internal, or subjective, dawn and CT12 with subjective dusk.

 $<sup>^{3}</sup>$ ZT (zeitgeber time) is used to describe the timing of an entraining agent and we associate ZT0 with the first occurrence of lights on (i.e. external dawn).

<sup>&</sup>lt;sup>4</sup>For a more in-depth discussion of  $\phi$  defined in the presence of perturbations, see (Brown et al., 2004).

<sup>&</sup>lt;sup>5</sup>The sIPRC is called the "infinitesimal PRC" or  $\mathbf{z}$  in much of the literature (Brown et al., 2004). In (Taylor et al., 2008) we named it the sIPRC to differentiate it from curves associated with parametric perturbation.

and can be interpreted as the infinitesimal phase shift  $(\partial \phi)$  resulting from a direct perturbation to the  $k^{th}$  entry of a solution along the limit cycle. More often, the sIPRC is used to predict the change in phase velocity, as part of a phase evolution equation:

$$\frac{d\phi(\mathbf{x}(t))}{dt} = 1 + \text{sIPRC}(\phi(\mathbf{x}(t))) \cdot \mathbf{S}(\phi(\mathbf{x}(t)), t)$$
(3)

where S is a vector of stimuli and is written as a function of either phase (i.e. the stimulus is from a clockcontrolled source) or time (i.e. the stimulus is dependent on zeitgeber time). Frequently, this model is used to capture oscillatory behavior of electrically stimulated neurons as they fire.

In earlier work (Taylor et al., 2008), we introduced analogous formulae for tracking the phase dynamics in the presence of *parametric* perturbation. The parametric impulse PRC (pIPRC) predicts the phase shift  $(\partial \phi)$  resulting from an infinitesimally short duration, infinitesimally small in magnitude perturbation to a parameter. The pIPRC for the *j*<sup>th</sup> parameter is defined

$$\operatorname{pIPRC}_{j}(\phi(\mathbf{x}(t))) = \frac{d}{dt} \frac{\partial \phi}{\partial p_{j}}(\phi(\mathbf{x}(t)))$$
(4)

$$= \frac{\partial}{\partial p_j} \frac{d\phi}{dt}(\phi(\mathbf{x}(t))) \tag{5}$$

$$= \operatorname{VRC}_{j}.$$
 (6)

From Eq. 5, it is clear that the pIPRC is also a velocity response curve – it predicts the change in phase velocity  $d\phi/dt$  due to a perturbation in parameter  $p_j$ . For the remainder of the present work, we refer to pIPRC<sub>i</sub> as VRC<sub>i</sub> or "the VRC."

To track the effects of parametric perturbation to component j over time, we rewrote the phase evolution equation using the VRC according to

$$\frac{d\phi(\mathbf{x}(t))}{dt} = 1 + \text{VRC}_j(\phi(\mathbf{x}(t)))s_j(\mathbf{x}(t), t)$$
(7)

where  $s_j(\mathbf{x}(t), t) = \Delta p_j(\mathbf{x}(t), t)$ .

The VRC is related to the sIPRC according to

$$VRC_{j}(\mathbf{x}(t)) = \sum_{k=1}^{N} sIPRC_{k}(\mathbf{x}(t)) \frac{\partial f_{k}}{\partial p_{j}}(\mathbf{x}(t))$$
$$= sIPRC \cdot \frac{\partial \mathbf{f}}{\partial p_{j}}$$

where N is the number of states.

The stimuli in the phase evolution equations are then related according to

$$S_i(\phi(\mathbf{x}(t)),t) = \frac{\partial f_i}{\partial p_j}(\phi(\mathbf{x}(t))) \ s_j(\phi(\mathbf{x}(t)),t),$$

and Eqs. 3 and 7 are related by:

$$\frac{d\phi(\mathbf{x}(t))}{dt} = 1 + \text{sIPRC}(\phi(\mathbf{x}(t))) \cdot \mathbf{S}(\phi(\mathbf{x}(t)), t)$$
$$= 1 + \text{sIPRC}(\phi(\mathbf{x}(t))) \cdot \frac{\partial \mathbf{f}}{\partial p_j}(\phi(\mathbf{x}(t))) s_j(\phi(\mathbf{x}(t)), t)$$
$$= 1 + \text{VRC}_j(\phi(\mathbf{x}(t))) s_j(\phi(\mathbf{x}(t)), t).$$

## **Gating Light**

To accurately reproduce light response data, we must accurately model the signal as it is seen by the clock. Experimental evidence indicates that light is gated by a "saturation" gate and a phase-dependent gate (which creates the deadzone) (Roenneberg et al., 2003; Comas et al., 2007; Comas et al., 2008). Thus, our model must incorporate such gates. In Figure 2 we show a conceptual picture of the clock and the input pathway of light. L(t) is the level of light in the environment. It passes through an initial gate  $G_I$ , which attenuates L(t) due to saturation and adaptation. The signal leaving gate  $G_I$  then passes through the phase-dependent gate  $G_P$  and finally into the core clock X, where it activates the transcription of *Per*.

We model the system shown in Figure 2, using a published model of the circadian clock as the core oscillator. The initial gate  $G_I$  mimics the response reduction and restoration dynamics observed in (Comas et al., 2006; Comas et al., 2007). Comas et al. (2006) reported a 78% attenuation in the response to light arriving after the first hour of a pulse. It is an open question as to the response during the first hour<sup>6</sup>, but

<sup>&</sup>lt;sup>6</sup>The response to short pulses of light has been studied for hamsters and mice (see, for example, (Nelson and Takahashi, 1999)),



Figure 2: Input Schematic. Light gates are shown as rectangles, the core clock as a circle, and input and feedback are shown as arrows. The external light cue *L* passes through the initial gate, which produces an attenuated light signal  $G_I(L)$ . This signal then passes through the phase-dependent gate, which uses clock components  $X_{GC}$  to compute further signal attenuation. The result is  $G_P(G_I(L), X_{GC})$ , which manipulates the core clock *X*. The core clock sends output signals to peripheral oscillators which, in turn, may feed back to the clock. The output processes are shown in gray and are not included in the models under consideration.

Fig. 5 in (Comas et al., 2007) suggests there is no significant attenuation.<sup>7</sup> We make the assumptions that (a) there is no attenuation during the first hour, and (b) the attenuation of the phase response is directly proportional to the attenuation in the signal. Thus, we model the initial gate such that it allows all light through for the first hour. Thereafter, it allows exponentially less light through, asymptoting at 22%. In the dark, the system restores its response capabilities according to an exponential curve. For the restoration, we use the exponential curve estimated by Comas et al. (2007).

We model the initial gate  $G_I$  as a function of light L and gate variable G. G has an upper steady-state  $G_{ss} > 1$  and a lower steady-state 0.22. When  $G \ge 1$ , all light is allowed through. Otherwise, it is attenuated according to

$$G_I(t,L) = \min(G(t), 1)L.$$
(8)

G is designed to travel from  $G_{ss}$  to 1 in the first hour of a light signal. It then decreases to within 5% of its

some with results indicating that light effects saturate at 15 minutes (Khammanivong and Nelson, 2000), but because these light pulses were much more intense (4960 lux) than those in Comas et al (2006; 2007) (100 lux), we model results in Comas et al. (2006; 2007) only.

<sup>&</sup>lt;sup>7</sup>Fig. 5 (Comas et al., 2007) shows a linear increase in the response for pulses up to 1 hour long (the first pulse of a two-pulse experiment). A linear relationship between the pulse duration and the magnitude of the response indicates no attenuation.

lower steady-state 0.22 in 0.8 hours. In darkness, G recovers its magnitude until it reaches  $G_{ss}^{8}$ . The ODE for G is

$$\frac{dG}{dt} = \begin{cases} \ln(0.005)(G - 0.22), & L = 0 \text{ (Dark)} \\ 0, & L > 0 \text{ and } G > G_{ss} \text{ (Light)} \\ 9.9344, & L > 0 \text{ and } G \le G_{thresh} \text{ (Light)}, \end{cases}$$
(9)

where  $G_{ss} = 0.78e^{-\ln(0.005)} + 0.22$ .

Geier et al. (2005) incorporated a phase-dependent gate into a model of the mouse clock (Becker-Weimann et al., 2004) by assuming that some (possibly not modeled) clock components are cycling in phase with modeled components. These unknown components interact with the pathway of light within the cell, preventing the signal from increasing the transcription of *Per* during the subjective day. We construct a similar gate, using a function bounded between 0 (no increase allowed) and 1 (the signal is ungated).

Because the trace of the gate-controlling clock component(s)  $X_{GC}$  is not known, we assume it is a linear combination of the modeled components (i.e. the state variables in the core clock model)

$$X_{GC}(\mathbf{x}) = \gamma \sum_{i=1}^{N} \alpha_i x_i.$$
<sup>(10)</sup>

The gate uses a function Y designed to allow maximal light through when  $X_{GC}$  is at its peak and to block passage of light when at its trough, i.e.

$$Y(X_{GC}) = \begin{cases} 0, & X_{GC} < A \\ \frac{1}{C} \left( \frac{1}{6} X_{GC}^3 - \frac{A+B}{4} X_{GC}^2 + \frac{AB}{2} X_{GC} + \frac{A^3}{12} - \frac{A^2B}{4} \right), & A \le X_{GC} \le B \\ 1, & X_{GC} > B. \end{cases}$$
(11)

where  $C = -\frac{B^3}{12} + \frac{AB^2}{4} - \frac{A^2B}{4} + \frac{A^3}{12}$  and *A* and *B* bound an interval between the minimum and maximum value of the  $X_{GC}$  oscillation, i.e.  $\min_t(X_{GC}(t)) \le A < B \le \max_t(X_{GC}(t))$ . The amount of light allowed through the phase dependent gate is then

$$G_P(t, L, X_{GC}) = G_I(t, L) \cdot Y(X_{GC}).$$
(12)

We assume that the gated signal increases the rate of Per transcription as an additive term, i.e. if the

<sup>&</sup>lt;sup>8</sup>Although the recovery of G is modeled as linear, the exponential saturation causes the effects of light to follow the exponential restoration curve published in (Comas et al., 2007).

state associated with nuclear Per mRNA is named MP, then its ODE would look like

$$\frac{dMP}{dt} = r_{MP} - d_{MP} + G_P(t, L, X_{GC}), \qquad (13)$$

where  $r_{MP}$  and  $d_{MP}$  are the terms representing regulation (e.g. by BMAL1) and degradation, respectively<sup>9</sup>.

We create a VRC to gated light,  $VRC_{GP}$ , with the deadzone, delay zone, and advance zone at the desired subjective times and with the proper magnitudes. These features are introduced by fitting *Y*'s parameters ( $\alpha_i$  and  $\gamma$ ). The process is straightforward if we use the concept of a *base* VRC – the VRC associated with the effects of light *without* the presence of a phase-dependent gate. Without the phase-dependent gate, Eq. 13 is

$$\frac{dMP}{dt} = r_{MP} - d_{MP} + G_I(t,L).$$
(14)

The light regulation term  $G_I(t,L)$  is a time-varying parameter, and its VRC, VRC<sub>GI</sub>, is our base VRC. Notice that  $\frac{d}{dG_I}\frac{dMP}{dt} = 1$ . If MP is the  $k^{th}$  state, then we can express this more generally as  $\frac{d}{dG_I}f_k = 1$ . This means that

$$\frac{df_k}{dG_I} = \begin{cases} 1, & \text{state } x_k \text{ represents } Per \text{ mRNA} \\ 0, & \text{state } x_k \text{ represents any other species} \end{cases}$$

Recall that the VRC is simply the dot-product of the vector of sIPRCs and the partial derivative  $\partial \mathbf{f}/\partial p_j$  (Eq. 8). For a model with only one light-activated *Per* gene, we have the base VRC

$$\operatorname{VRC}_{GI}(\phi) = \operatorname{sIPRC}_k(\phi),$$

where  $x_k$  is the state associated with *Per* mRNA. For a model with more than one light-activated *Per* gene, the base VRC is simply the sum of the sIPRCs for the *Per* mRNA states.

To fit the phase-dependent gate parameters, we minimize the distance between an ideal VRC,  $VRC_{ideal}$ , and  $VRC_{GP}$  where

$$\operatorname{VRC}_{GP}(\phi) = \operatorname{VRC}_{GI}(\phi)Y(\phi). \tag{15}$$

<sup>&</sup>lt;sup>9</sup>For the majority of studies of light affecting the oscillator, light is incorporated as an additive term. One exception is in (Leloup and Goldbeter, 2004), in which the authors increase the maximal rate of transcription within the regulation term itself. In this case, the regulation by BMAL1 is the phase-dependent gate.

## **Phase Response Curves**

To compute the phase shift resulting from a light signal L(t) with onset at phase  $\phi_1$ , we employ one of the following methods:

• Full Model Method – Perform a numerical experiment with the full model. Compare the position of a marker (such as the peak of *Per* mRNA) in the reference trajectory to that in a simulation with a *w*-hour light pulse. For example, if the two trajectories share the same initial conditions and the experimental trajectory returns to the limit cycle by the tenth cycle, then we compute the phase shift using  $t_{ref}$  (the time of the peak of *Per* mRNA in the tenth cycle of the reference trajectory) and  $t_{exp}$  (the time of the peak in the tenth cycle of the experimental trajectory) according to

$$PRC(\phi_1) = t_{exp} - t_{ref}.$$
(16)

• Phase Evolution Method – Solve the phase evolution equation (Eq. 7) in the presence of the light pulse. Compare the phase to zeitgeber time at the end of the simulation, according to

$$PRC(\phi_1) = \phi(t_{end}) - t_{end}.$$
(17)

• Method of Averaging – Use the VRC with the method of averaging. Compute the PRC<sup>10</sup> with  $G_I(t,L)$ and VRC<sub>GP</sub> via

$$PRC(\phi_1) = \int_0^\tau VRC_{GP}(t+\phi_1)G_I(t,L)dt.$$
(18)

For more details, see (Taylor et al., 2008).

## Entrainment

#### **Phase Angle of Entrainment**

The phase angle of entrainment relates the phase of the clock to the zeitgeber, e.g. the dawn phase angle of entrainment is the phase of the entrained clock when it encounters the onset of light each day. Given a light/dark pattern *LD*, where the photoperiod lasts *w* hours, the phase angle of entrainment  $\psi$  is predicted

<sup>&</sup>lt;sup>10</sup>Computing the PRC with the method of averaging is similar to calculating the interaction function H in (Kuramoto, 1984).

by the PRC. For a natural photoperiod, we compute the dawn phase angle of entrainment  $\psi_{BL}$  and the dusk phase angle of entrainment  $\psi_{EL}$ .

- If PRC( $\phi$ ) is the phase shift incurred by a light pulse *beginning* at phase  $\phi$  and lasting *w* hours, then  $\psi_{BL}$  is the phase such that PRC( $\psi_{BL}$ ) =  $\tau T$  and the slope of the PRC is negative at  $\psi_{BL}$ .
- If  $PRC_{offset}(\phi)$  represents the phase shift incurred by a *w*-hour light pulse *ending* at phase  $\phi$ ,  $\psi_{EL}$  is the phase such that  $PRC_{offset}(\psi_{EL}) = \tau T$  and the slope of the PRC is negative at  $\psi_{EL}$ . According to limit cycle theory, phase adjustments are made while the clock is receiving a signal and end when the signal ends. The state trajectories may still be in transient, but the phase shifting has completed. Thus the phase of pulse offset must take into account both the duration of the pulse and the shift it incurs. The offset PRC is computed from the onset PRC according to

$$PRC_{offset}(\phi + w + PRC(\phi)) = PRC(\phi)$$
, for all  $\phi$ .

#### **Phase Transition Map**

The phase transition curve (PTC) describes the phase of the oscillator one cycle after encountering a pulse of light. It is computed from the PRC for the appropriate pulse of light according to

$$PTC(\phi) = \phi + PRC(\phi).$$

The phase transition map (PTM) describes the phase of the next cycle, but with respect to an entraining signal of period T. It is computed from the PTC according to

$$PTM(\phi) = PTC(\phi) + T - \tau.$$
(19)

The phase angle of entrainment  $\psi$  falls on the intersection of the PTM and the line  $\phi = \phi$ .

#### A Closer Look at the VRC Theory of Entrainment

The VRC theory of entrainment states that the rate of internal time (i.e. phase progression) is adjusted continuously, but that most of the adjustment occurs circa dawn and dusk (Swade, 1969; Daan and Pittendrigh, 1976b). Figure 1A shows a theoretical VRC plotted as a function of phase. The response to light is an adjustment in the speed – during early subjective morning the clock will speed up, during subjective daytime there is a deadzone with no response, during early subjective evening the clock will slow down, and during the subjective night the clock will transition from deceleration to acceleration. In the presence of an entraining light signal, the phase is dynamically adjusting and the VRC is deformed (as is shown for PRCs by Pittendrigh and Daan (1976) Figure 2).

The VRC illustrates both the differences and similarities between the continuous model of entrainment and the discrete model. Figures 1B and 1C show the VRC as a function of zeitgeber time of fast clock  $(\tau = 23.7h)$  under full photoperiod (LD12:12) and photoskeleton (LD1:10:1:23) entrainment, respectively. The dawn and dusk phase angles of entrainment are nearly identical despite the different light schedules (in Figures 1B and C, the VRC values at ZT0 and ZT12 are very close). However, examining the VRC values throughout the entire daytime reveals the subtle differences in the phase dynamics leading to those phase angles. In the presence of 12 hours of light, the dynamics are shifted more gradually, most notably during the period between ZT8 and ZT12. Figure 1D allows us to track the phase dynamics more precisely, plotting the difference between the clocks' phases and zeitgeber time. At ZTO, the phase of the system undergoing full photoperiod entrainment is approximately CT0.1 and the phase of system undergoing photoskeleton entrainment is approximately CT23.4. Under both scenarios, the phase is adjusted dramatically in the first hour of light. During the daytime, the phases gradually drift away from zeitgeber time - the slope is positive because the clock represented here has a short period. The system undergoing full photoperiod entrainment is adjusted during the last 4 hours of daylight while the system undergoing photoskeleton entrainment is adjusted only during the dusk light pulse. Thus we see clearly that the continuous model of entrainment can account for data observed in photoskeleton experiments. As long as there is a deadzone during the day, it can be difficult to discern the difference between continuous and discrete entrainment in short-pulse entrainment experiments.

The deadzone also plays an important role in conserving the phase angle of entrainment over different photoperiods. As in the discrete theory (Pittendrigh and Daan, 1976), the VRC theory holds that short-period clocks (those in nocturnal animals) conserve the dusk phase angle under different photoperiods and that long-period clocks (those in diurnal animals) conserve the dawn phase angle. Figure 1B illustrates how a short-period clock can conserve its dusk phase for photoperiods of 12 hours or less. In LD12:12, we see that the photoperiod ranges from just before the deadzone, across the deadzone, and well into the delay zone



Figure 3: Theoretical VRCs of varied shape allow for similar stable phases of entrainment under different photoperiods. Shown are theoretical VRCs for (A) short-period and (B) long-period clocks. For each VRC, a phase-only model was entrained to a 24-hour LD schedule using photoperiods of 8 to 16 hours. The stable dawn and dusk phase angles for (C) short-period and (D) long-period clocks are drawn with gray rectangles in the background. The gray rectangles indicate perfect photoperiodic behavior in which a short-period clock conserves a dusk phase of CT12 and a long-period clock conserves a dawn phase of CT0.

(see Figure 1B), allowing the clock to encounter overall delays. A shorter photoperiod must cause the same overall delay. Thus, shortening the photoperiod fixes dusk and causes dawn to move into the deadzone. For longer photoperiods the deadzone cannot accommodate all of the morning, so dawn must occur during the advance zone.

VRCs with a variety of shapes allow for near-conservation of dawn and dusk in long-period and sortperiod clocks, respectively. To illustrate, we choose eight theoretical VRCs with deadzones ranging in width from 3 to 8 circadian hours and with delay and advance zones with different characteristics (some are wide, some narrow, some VRCs favor delays, while others favor advances). Short-period ( $\tau = 23.3$ ) and long-period ( $\tau = 24.7$ ) clocks with each of these VRCs entrain to 24-hour cycles with photoperiods ranging from 8 to 16 hours. We show the VRCs and the dawn and dusk phase angles for a short-period clock in Figures 3A and 3C. In Figures 3B and 3D, we show the same for a long-period clock. The VRCs in the two upper panels are identical in shape, but are aligned to allow for the dawn phase angle of entrainment under LD12:12 to be CT0. Regardless of the precise shape of the VRC, short-period animals conserve the dusk phase (gaining at most 2.5 hours) and long-period animals conserve the dawn phase (losing at most 3.9 hours). Of the prominent characteristics (area under the delay and advance sections, slopes of the delay and advance sections, width of the advance, delay, and deadzones, and maximal delays and advances), only the deadzone width indicates the degree of phase angle conservation – the longer the deadzone, the better the conservation.

# Results

The ideal VRC to light will cross zero with positive slope in the subjective evening (circa CT15-CT18). The positive-slope zero-crossings of four published mammalian models are CT0.2 (Forger and Peskin, 2003), CT11.8 (Leloup and Goldbeter, 2003), CT15.6 (a modified Goodwin oscillator) (Gonze et al., 2005), and CT15.7 (Becker-Weimann et al., 2004). We incorporate both light gates into the latter two models, designing the phase-dependent gate to produce a VRC similar to that of the nocturnal animal model in (Geier et al., 2005). For the modified Goodwin oscillator, the phase dependent gate  $G_P$  (Eq. 12) uses  $X_{GC}(\mathbf{x}) = 0.5417x_2 + 0.9784x_3$ , A = 2.2155, and B = 2.4506. For the 7-state model of Becker-Weimann (2004),  $G_P$  (Eq. 12) uses  $X_{GC}(\mathbf{x}) = 0.2x_3$ , A = 1.5326, and B = 1.8701. As in (Geier et al., 2005), we scale the rate constants to acquire a free-running period  $\tau$  of 23.7 hours. We label the former model MGG (Modified Goodwin with Gate) and the latter BWG (Becker-Weimann with Gate).

The PRCs for these two models are computed for light pulses of durations 1, 3, 4, 6, 9, 12, and 18 hours, as in (Comas et al., 2006). Figure 4 shows the PRCs for BWG computed via A) the full model method, B) the phase evolution method, and C) the method of averaging. We align them according to the approximate circadian time of the center of the pulse (again, as in (Comas et al., 2006)). The results are similar for MGG (data not shown).

To evaluate the VRC theory of entrainment, we study the process of re-entrainment when the clock is out of phase with the environment. We perform numerical experiments mimicking those most commonly performed in behavioral experiments, i.e. single pulse entrainment, full photoperiod entrainment, and photoskeleton entrainment experiments. To begin, we attempt to entrain the clock models with a daily LD schedule of 1:23<sup>11</sup> and find that MGG does not entrain. Even after a stable phase of entrainment seems to be achieved, the amplitude of oscillations continues to vary. Thus for the remainder of the experiments "the model" refers to BWG. The entrainment experiments for this model use L = 1.5 to indicate "lights on" and are initiated from 25 initial conditions, covering the entire cycle (i.e. we associate the onset of light in zeitgeber time (ZT0) with CT0, CT1, CT2, etc.). We predict the stable phase of entrainment using the full model 1-hour PRC (Figure 5A, top panel) as  $\hat{\psi}_{BL}$  =CT11. All experiments converge to an actual stable phase angle of entrainment  $\psi_{BL}$ =CT10.7, with the time to convergence dependent upon the initial phase difference between circadian time and zeitgeber time. In the lower panel of Figure 5A, we show the number of cycles required to reset the clock to within 15 minutes of  $\psi_{BL}$  versus the circadian time associated with ZTO. To examine the process of entrainment, we plot the phase transitions for three experiments (ZTO associated with CT6, CT12, and CT18) on the phase transition map (PTM) computed from the full model PRC (Figure 5B). For each circle, its x-axis position is its phase at the onset of light ( $\phi_{BL}$ ) of cycle *i* and its y-axis position is its phase at the onset of light of cycle i + 1. The circles are connected to clarify the process from cycle to cycle. The clocks in the ZT0=CT6 and ZT0=CT18 experiments reach  $\psi_{BL}$  by advancing their phase daily, while the ZT0=CT12 experiment's clock delays its phase daily. Figure 5C summarizes the results of all experiments by showing the phase of the clock at the onset of light each day.

To examine the effects of a full photoperiod, we entrain the clock with an LD schedule of 12:12. The entrainment experiments are initiated from 25 initial conditions, covering the entire cycle (i.e. we associate ZT0 with CT0, CT1, CT2, etc.). We predict the stable phase of entrainment using the full model 12-hour PRC (Figure 6A, top panel) as  $\hat{\psi}_{BL}$ =CT1.3. Some experiments converge to an actual stable phase angle of entrainment  $\psi_{BL}$ =CT1.5, while others converge to  $\psi_{BL}$ =CT14. In the lower panel of Figure 6A, we show the number of cycles required to reset the clock to within 15 minutes of  $\psi_{BL}$  versus the circadian time associated with ZT0. The experiments converging to the predicted  $\psi_{BL}$  are shown with filled circles and those converging to  $\psi_{BL}$ =CT14 are shown with empty circles. Like above, we plot the phase transitions for three experiments (ZT0 associated with CT6, CT12, and CT18) on the PTM computed from the full model PRC (Figure 6B). The clocks in the ZT0=CT6 and ZT0=CT18 experiments reach the  $\psi_{BL}$  by advancing their phase daily, while the ZT0=CT12 experiment's clock delays its phase on the first day and advances it the

<sup>&</sup>lt;sup>11</sup>That, is a daily light/dark schedule of 1 hour of light followed by 23 hours of dark.

remaining days. We examine the state trajectories for all experiments and find that, for experiments converging to the predicted phase of entrainment, all entrained state trajectories are similar to their trajectories in constant darkness. In contrast, the experiments entraining to an incorrect phase show state trajectories deviating significantly from their cycle in constant darkness (data not shown).

To further investigate the entrainment process with a full photoperiod, we turn to a phase-only system in which we use the PTM directly to determine the daily changes in phase. As above, the entrainment experiments are initiated from 25 initial conditions and the PTM is computed from the full model 12-hour PRC. Figure 7A shows the 12-hour PRC and the time to convergence. Again we plot the phase transitions for three experiments (ZT0 associated with CT6, CT12, and CT18) on the PTM computed from the full model PRC (Figure 7B). The clocks in the ZT0=CT6 and ZT0=CT12 scenarios reach the  $\psi_{BL}$  by delaying their phase daily, while the ZT0=CT18 scenario's clock advances daily. Figure 7C summarizes the results.

To compare the full photoperiood to the skeleton photoperiod, we repeat the experiments for a LDLD schedule of 1:10:1:12. To predict the stable phase of entrainment, we compute a phase response curve (via the full model method) to a light pattern LDL=1:10:1. There are two theoretical stable phase angles of entrainment (Figure 8A) as  $\hat{\psi}_{BL}$  =CT23.7 (i.e. the first light pulse is associated with dawn) and CT11 (i.e. the second light pulse is associated with dawn). In Figure 8B, we show the number of cycles required to reset the clock to within 15 minutes of  $\psi_{BL}$  versus the circadian time associated with ZTO. Experiments converge to  $\psi_{BL}$  =CT23.4 (filled circles) and to  $\psi_{BL}$ =CT10.7 (empty circles). As with the 1-hour single pulse entrainment experiments, the system remains on the PTM throughout the process of entrainment (data not shown).

Finally, we study the dawn and dusk phases of entrainment under 24-hour LD cycles with photoperiods of 8, 9, 10, 11, 12, 13, 14, 15, and 16 hours. First, using a phase-only model (Eq. 7) with the full model's VRC (shown in Figure 9A), we predict the dawn and dusk phase angles. The predicted dawn angles  $\psi_{BL}$  are CT5.7, CT4.7, CT3.6, CT2.6, CT1.6, CT0.53, CT23.5, CT22.5, and CT21.4, respectively. The predicted dusk angle  $\psi_{EL}$  is CT13.2 for all photoperiods. Next, we use the full model PRCs for 8- to 16-hour pulses of lights. We predict  $\psi_{BL}$  will be CT5.3, CT4.3, CT3.3, CT2.2, CT1.2, CT0.2, CT23.5, CT22.2, and CT21.2 (see Figure 9B). The dusk phase angles  $\psi_{EL}$  are predicted to be CT12.9 for photoperiods of 8 to 13 hours and CT13 for photoperiods of 14 to 16 hours (see Figure 9C). After entraining the full model, the observed dawn phase angles are CT5.6, CT4.5, CT3.5, CT2.5, CT1.5, CT0.4 CT23.4, CT22.4, and CT21.3, respectively. The dusk phase angle is always CT13.

# Discussion

The gated Becker-Weimann model entrains to LD schedules with a stable wave form and is the model we consider for the remainder of paper, refering to it as "the model." Its PRCs (computed with the full model method) closely match the data in (Comas et al., 2006). The trends in phase evolution and averaging predictions are correct as well. Together these data show that modeling the correct velocity response curve is, at least for this model, sufficient for predicting the longterm response to differing light signals. This is significant because the PRC shapes change as the duration of light increases. The model PRC displays an increase in the delay to advance ratio. As is typical for a nocturnal animal, the PRC to a one-hour light pulse produces an area under the delay region, D, greater than the area under its advance region, A. Comas et al. observed that |A - D| grew with pulse duration and postulated that the reverse might be seen in PRCs collected for typical diurnal animals (i.e. A > D in the 1-hour PRC). With the method of averaging, it is relatively straight forward to show that this should be the case and that A - D is proportional to  $w \cdot \int_0^\tau VRC(\phi) d\phi$ . The agreement between full model PRCs and phase-only PRCs also support conclusions drawn from previously published phase-only models (Comas et al., 2006; Comas et al., 2007) that longerduration PRCs can be predicted from short-duration pulse PRCs. Here, we are assuming not that the 1-hour PRC is the basis for computation, but that the VRC, an infinitesimally short pulse PRC, is the basis for computation.

Our simulations show that the steady-state response of the model is biologically realistic, but that this is no guarantee that it will re-entrain properly from all initial phase mismatches. In other words, demonstrating the correct long-term response is not equivalent to demonstrating the correct short-term (24-hour) response. The full model shows a realistic short-term response and re-entrains to the correct phase angle  $\psi_{BL}$  if the model returns promptly to the DD limit cycle during each scotoperiod. For the 9 full photoperiod simulations that result in an incorrect  $\psi_{BL}$ , the entrained cycle differs significantly from the DD limit cycle.

After a 1-hour pulse of light, the model does return to the DD limit cycle, and simulations using these pulses are realistic on several levels. First, all methods of 1-hour pulse PRC computation produce nearly identical *in silico* experimental PRCs, which are, in turn, very similar to *in vivo* (behavioral) PRCs. Second, the numerical experimental entrainment process flows according to the theoretical PTM, a process which, again, is a reasonable description of the process of entrainment seen in behavioral experiments. The data in Figure 5 provide additional support for the hypothesis of Watanabe et al. (2001) that resetting is accom-

plished within the first day even when the output indicates a lag. In addition, it supports conclusions from two-pulse experiments such as those by Best et al. (1999), which indicate that resetting is accomplished within two hours. In our data it is clear that the phase shift is completed before the end of the cycle at all sections of the curve; the data from all cycles in all experiments aligns with the PTM (see Figure 5B; three are shown, but the statement is true for all).

In natural light/dark cycles, we expect to see phase velocity increase in the early morning, no adjustments made during the deadzone, and then phase velocity decrease in the early evening. The overall phase adjustment is captured by the 12-hour PRC which should then be used to predict the process of entrainment from cycle to cycle. The 12-hour PRC is used to compute the PTM for the full photoperiod entrainment simulations. Our data show that the PTM consistently predicts realistic re-entrainment (see Figure 7), but that the full model simulations are realistic only when the model returns quickly to the DD limit cycle. For entrainment simulations beginning in the ranges CT0 to CT6 and CT16 to CT24, the full model simulations return to the DD limit cycle relatively quickly, follow the PTM, and entrain realistically. For example, these data show a correct phase angle of entrainment and converge within a week (Yamazaki et al., 2000). It is significant that the process of re-entrainment from an animal/environment mismatch follows the PTM because it demonstrates the ability of the clock to be adjusted continuously by light repeatedly and for that action to be predictable and effective at re-entrainment. This provides direct support for the VRC dictating phase response behavior of the clock and the contention that dawn and dusk light transitions play no special role in entrainment. This is consistent with recent step-PRC experiments, the results of which can be sufficiently explained by the continuous theory, but not by the discrete (Comas et al., 2008).

The relationship between the discrete and continuous theories can be further understood by comparing PRCs and entrainment for full photoperiods to those for skeleton photoperiods. Both the 12-hour PRC (Figure 7A, upper panel) and the two-pulse 1:10:1 PRC (Figure 8A) predict dawn stable phase angles of entrainment relatively close to CT0 (CT23.7 for the photoskeleton and CT1.3 for the photoperiod). This is readily explained by presence of a deadzone in the VRC – if light is unable to change the clock's velocity during the middle of the daytime, then we expect the phase of entrainment to be similar whether or not light is actually shone on the clock in the middle of the day. The difference in photoskeleton and photoperiod entrainment is seen in experiments beginning from large mismatches between mouse and environment phases. Only the photoskeleton PRC predicts two stable phase angles, one in which the 10-hour period of darkness is considered the "daytime" and the other in which the 12-hour period of darkness is considered the "daytime." This is consistent with behavioral experiments (Pittendrigh and Daan, 1976). Thus, despite the predictive power of the skeleton photoperiod, the full photoperiod is required to ensure that the clock re-entrains in such a way that *Per* mRNA peaks during the day.

The relationship between the VRC, the free-running period, and the zeitgeber predicts the stable phase of entrainment. Our simulations entrain such that the dawn and dusk phases of entrainment are accurately predicted by the VRC-based phase-only model. First, the free-running period is short, which means we expect the dusk phase of entrainment to be conserved over changing photoperiods. The deadzone for this model is 14 hours wide, allowing it to conserve the dusk phase angle not just over short photoperiods, but over long photoperiods as well. Figure 9A demonstrates the phase relationship between photoperiods of increasing duration and the clock. All photoperiods encounter the same section of the delay zone, and extend as far as necessary into the deadzone. This highly intuitive behavior is also predicted by PRCs to long pulses of light (see Figures 9B and 9C). A long deadzone allows a short-period clock to perfectly conserve its dusk phase of entrainment – a feature advantageous to a nocturnal mammal needing to forage at dusk. When only approximate conservation of dusk is necessary, a deadzone as short as 3 hours may be sufficient (see Figure 3).

In summary, discrete and continuous entrainment are unified by the VRC and can be studied using both the VRC and the PRC to long pulses of light. Further, much of the intuition developed under the discrete theory can be transferred directly to the continuous theory. For example, straight-forward prediction of the phase angle of entrainment – a hallmark of the discrete theory – is also possible within the VRC-unified theory. In addition, we have shown that the presence of a deadzone is important both functionally and theoretically. Functionally, the deadzone allows short-period clocks to conserve the dusk phase angle of entrainment. Theoretically, examining the deadzone reveals why it is difficult to distinguish between continuous and discrete effects using short pulses of light (see Figure 1). Finally, the implication of this work is not only a call for more experimentation with long light pulses (such as those of similar to those of Comas et al. (2006; 2007; 2008)) but also a re-examination of short-pulse experiments in light of continuous theory. Collection of a VRC would be ideal, but is not straight-forward. However, we suggest phase-only modeling and careful attention to light gating be used to uncover a good approximate VRC.

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# **Figure Legends**

**Figure 1.** Velocity response curves (VRCs) in free-run and under entrainment. A) The VRC has a shape similar to that of a PRC, but shows changes in the clock's velocity. When the VRC is one, then light (L = 1) doubles the speed of the clock. The VRC is shown as its changes under B) full photoperiod (12:12) and C) skeleton photoperiod (1:10:1:12) entrainment. D) Shown is the difference between zeitgeber time and the phase of the clock under both entrainment scenarios (solid line for the full photoperiod, dashed line for the skeleton photoperiod). Positive values indicate the internal phase is ahead of zeitgeber time. The dark gray background indicates night in both scenarios. The light gray background from ZT1 to ZT11 indicates the period of darkness during photoskeleton entrainment.

**Figure 2.** Input Schematic. Light gates are shown as rectangles, the core clock as a circle, and input and feedback are shown as arrows. The external light cue *L* passes through the initial gate, which produces an attenuated light signal  $G_I(L)$ . This signal then passes through the phase-dependent gate, which uses clock components  $X_{GC}$  to compute further signal attenuation. The result is  $G_P(G_I(L), X_{GC})$ , which manipulates the core clock *X*. The core clock sends output signals to peripheral oscillators which, in turn, may feed back to the clock. The output processes are shown in gray and are not included in the models under consideration.

**Figure 3.** Theoretical VRCs of varied shape allow for similar stable phases of entrainment under different photoperiods. Shown are theoretical VRCs for (A) short-period and (B) long-period clocks. For each VRC, a phase-only model was entrained to a 24-hour LD schedule using photoperiods of 8 to 16 hours. The stable dawn and dusk phase angles for (C) short-period and (D) long-period clocks are drawn with

gray rectangles in the background. The gray rectangles indicate perfect photoperiodic behavior in which a short-period clock conserves a dusk phase of CT12 and a long-period clock conserves a dawn phase of CT0.

**Figure 4.** Phase Response Curves for the Becker-Weimann model with both gates. PRCs calculated with A) the full model method, B) the phase evolution method, and C) the method of averaging are shown for light pulses of durations 1, 3, 4, 6, 9, 12, and 18 hours. The phase shift is plotted as a function of the circadian time of the middle of the pulse.

**Figure 5.** Full Model Entrainment of BWG by a 1:23 LD schedule. A) The PRC to a 1-hour pulse of light is shown in the upper panel, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangle shows the predicted stable phase angle of entrainment (with respect to pulse onset). The bottom panel shows the number of cycles required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. the onset ZT0 of the first light pulse). B) The phase transitions are shown for three entrainment experiments – ZT0=CT6 for cycle 1 (upper panel), ZT0=CT12 for cycle 1 (middle panel), and ZT0=CT18 for cycle 1 (lower panel). For all three, the dotted line represents the theoretical PTM, the filled triangle is the predicted stable phase angle of entrainment, the black filled circle is the actual stable phase angle of entrainment, the gray circles represent the phase transitions for cycles 1 to 50 of the experiment, and the arrows indicate the general direction of the phase shifts. C) The process of entrainment is shown for 25 experiments with the circadian time of dawn shown for each of the 50 cycles.

**Figure 6.** Full Model Entrainment of BWG by a 12:12 LD schedule. A) The PRC to a 12-hour pulse of light is shown in the upper panel, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangle shows the predicted stable phase angle of entrainment. The bottom panel shows the number of cycles required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. Experiments converge to the predicted phase of entrainment (circa CT0; filled circles) and to one approximately in anti-phase (circa CT12; empty circles). The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. ZT0). B) Theoretical phase transitions are shown for three entrainment experiments – ZT0=CT6 for cycle 1 (upper panel), ZT0=CT12 for cycle 1 (middle panel), and ZT0=CT18 for cycle 1 (lower panel). For all three, the dotted line represents the theoretical PTM, the filled triangle is the predicted stable phase angle of entrainment, the gray circles represent the phase transitions for cycles 1 to 50 of the experiment, and the arrows indicate the general direction of the phase shifts. C) The process of entrainment

is shown for 25 PTM-based simulations with the circadian time of dawn shown for each of 50 cycles.

**Figure 7.** PTM Entrainment by a 12:12 LD schedule. A) The PRC to a 12-hour pulse of light is shown in the upper panel, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangle shows the predicted stable phase angle of entrainment. The bottom panel shows the number of cycles required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. the onset ZT0 of first light pulse). B) The phase transitions are shown for three entrainment scenarios – ZT0=CT6 for cycle 1 (upper panel), ZT0=CT12 for cycle 1 (middle panel), and ZT0=CT18 for cycle 1 (lower panel). For all three, the dotted line represents the theoretical PTM, the filled triangle is the predicted stable phase angle of entrainment, the gray triangles represent the phase transitions for cycles 1 to 50 of the experiment, and the arrows indicate the general direction of the phase shifts. C) The process of entrainment is shown for 25 scenarios with the circadian time of dawn shown for each of 50 cycles.

**Figure 8.** Entrainment of BWG by a 1:10:1:12 LDLD schedule. A) The PRC to two 1-hour pulses of light (separated by 10 hours of darkness) is shown, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangles show the two predicted stable phase angle of entrainment (with respect to the onset of the first pulse). B) Shown are the number of cycles each experiment required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. ZT0).

**Figure 9.** Entrainment under photoperiods of 8 to 16 hours. (A) The model's VRC is double-plotted. The photoperiods (all ending at CT13) are shown in gray rectangles, with lighter grays for longer photoperiods. (B) The full model PRCs for 8- to 16-hour pulses of light are shown, aligned according to the time of the pulse onset. The ranges of the predicted dawn phase angles  $\psi_{BL}$  are indicated with arrows. (C) The full model PRCs for 8- to 16-hour pulses of light are aligned according to the time of pulse offset. The predicted dawn phase angles  $\psi_{BL}$  are indicated with arrows. (C) The full model PRCs for 8- to 16-hour pulses of light are aligned according to the time of pulse offset. The predicted dusk phase angle  $\psi_{EL}$  is indicated with an arrow.



Figure 4: Phase Response Curves for the Becker-Weimann model with both gates. PRCs calculated with A) the full model method, B) the phase evolution method, and C) the method of averaging are shown for light pulses of durations 1, 3, 4, 6, 9, 12, and 18 hours. The phase shift is plotted as a function of the circadian time of the middle of the pulse.



Figure 5: Full Model Entrainment of BWG by a 1:23 LD schedule. A) The PRC to a 1-hour pulse of light is shown in the upper panel, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangle shows the predicted stable phase angle of entrainment (with respect to pulse onset). The bottom panel shows the number of cycles required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. the onset ZT0 of the first light pulse). B) The phase transitions are shown for three entrainment experiments – ZT0=CT6 for cycle 1 (upper panel), ZT0=CT12 for cycle 1 (middle panel), and ZT0=CT18 for cycle 1 (lower panel). For all three, the dotted line represents the theoretical PTM, the filled triangle is the predicted stable phase angle of entrainment, the black filled circle is the actual stable phase angle of entrainment, the gray circles represent the phase transitions for cycles 1 to 50 of the experiment, and the arrows indicate the general direction of the phase shifts. C) The process of entrainment is shown for 25 experiments with the circadian time of dawn shown for each of the 50 cycles.



Figure 6: Full Model Entrainment of BWG by a 12:12 LD schedule. A) The PRC to a 12-hour pulse of light is shown in the upper panel, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangle shows the predicted stable phase angle of entrainment. The bottom panel shows the number of cycles required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. Experiments converge to the predicted phase of entrainment (circa CT0; filled circles) and to one approximately in anti-phase (circa CT12; empty circles). The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. ZT0). B) Theoretical phase transitions are shown for three entrainment experiments – ZT0=CT6 for cycle 1 (upper panel), ZT0=CT12 for cycle 1 (middle panel), and ZT0=CT18 for cycle 1 (lower panel). For all three, the dotted line represents the theoretical PTM, the filled triangle is the predicted stable phase angle of entrainment, the gray circles represent the phase transitions for cycles 1 to 50 of the experiment, and the arrows indicate the general direction of the phase shifts. C) The process of entrainment is shown for 25 PTM-based simulations with the circadian time of dawn shown for each of 50 cycles.



Figure 7: PTM Entrainment by a 12:12 LD schedule. A) The PRC to a 12-hour pulse of light is shown in the upper panel, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangle shows the predicted stable phase angle of entrainment. The bottom panel shows the number of cycles required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. the onset ZT0 of first light pulse). B) The phase transitions are shown for three entrainment scenarios – ZT0=CT6 for cycle 1 (upper panel), ZT0=CT12 for cycle 1 (middle panel), and ZT0=CT18 for cycle 1 (lower panel). For all three, the dotted line represents the theoretical PTM, the filled triangle is the predicted stable phase angle of entrainment, the gray triangles represent the phase transitions for cycles 1 to 50 of the experiment, and the arrows indicate the general direction of the phase shifts. C) The process of entrainment is shown for 25 scenarios with the circadian time of dawn shown for each of 50 cycles.



Figure 8: Entrainment of BWG by a 1:10:1:12 LDLD schedule. A) The PRC to two 1-hour pulses of light (separated by 10 hours of darkness) is shown, plotted with the phase shift as a function of the circadian time of the onset of the pulse (ZT0). The filled triangles show the two predicted stable phase angle of entrainment (with respect to the onset of the first pulse). B) Shown are the number of cycles each experiment required to converge to (i.e. remain within 0.25 hours of) the stable phase of entrainment. The x-axis represents the circadian time of the clock when the entrainment experiment begins (i.e. ZT0).



Figure 9: Entrainment under photoperiods of 8 to 16 hours. (A) The model's VRC is double-plotted. The photoperiods (all ending at CT13) are shown in gray rectangles, with lighter grays for longer photoperiods. (B) The full model PRCs for 8- to 16-hour pulses of light are shown, aligned according to the time of the pulse onset. The ranges of the predicted dawn phase angles  $\psi_{BL}$  are indicated with arrows. (C) The full model PRCs for 8- to 16-hour pulses of light are aligned according to the time of pulse offset. The predicted dusk phase angle  $\psi_{EL}$  is indicated with an arrow.