Dynamics of a Multistage Circadian System

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Abstract Tissues throughout the body exhibit circadian rhythms, forming a multioscillatory system whose disruption results in jet lag and other health problems in travelers and rotational shift workers. The authors' simulations of the dynamics of a multistage circadian system (based on experimental results for nocturnal rodents) reveal the flexibility and stability inherent in a multistage system, as well as potential pitfalls. The modeling predicts that jet lag tends to be most severe following an eastward change of 5 to 8 time zones due to prolonged desynchrony of the system. This desynchrony is partly due to differing reentrainment rates among components, but a much greater source of desynchrony is the antidromic reentrainment of some but not all components (reentrainment by partition), triggered by the overshoot of the master pacemaker's phase in response to these advances. Based on the multistage system dynamics, the authors design a simple protocol that results in a more orderly transition that avoids antidromic reentrainment in all components, thereby reducing the reentrainment time from nearly 2 weeks to just a few days for the most difficult shifts. The authors compare the predicted behavior of self-sustaining versus damped oscillatory components in the system as well as the effect of weak versus strong coupling from the master pacemaker to the peripheral components.

Key words SCN, mathematical modeling, circadian rhythms, multiple circadian oscillators, entrainment, phase shifts, jet lag

The circadian system maintains a 24-h rhythm that controls many physiological processes. It entrains to a regular schedule, allowing organisms to anticipate and prepare for the usual activities of the day, thereby possibly conferring an evolutionary advantage (Dodd et al., 2005). Chances of survival and quality of life are likely increased by the multistage structure of the circadian system, which integrates environmental cues with the endogenous clock to create a daily activity pattern (Saper et al., 2005). However, desynchronization of the circadian system in humans may occur after the abrupt schedule changes that have become common in modern society, due to rotational shift work and rapid travel across multiple time zones. This temporary desynchrony probably contributes to a variety of "jet lag" symptoms, from loss of alertness to gastric distress, as well as increased risk of cancer and coronary heart disease in nurses who regularly rotate shifts and airline attendants who frequently cross time zones (Mawson, 1998; Learthart, 2000; Davis et al., 2001; Klerman, 2005). In this article, we explore a model of the circadian system of the rat as a first step in better understanding the transient dynamics of jet lag from a system point of view.

The master pacemaker of the mammalian circadian system is the SCN in the hypothalamus (Alvarez, 2004). Many other tissues also exhibit circadian

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oscillations (Oishi et al., 1998; Le Minh et al., 2001; Stokkan et al., 2001; Balsalobre, 2002; Storch et al., 2002; Yoo et al., 2004). For example, lung, liver, and skeletal muscle tissues of transgenic rats exhibit rhythmic expression of *Period1* for several days in vitro (Yamazaki et al., 2000). The SCN responds to light and can be entrained by the LD cycle (dawn and dusk "set the clock" in the SCN) and, in turn, entrains other oscillatory tissues in the body.

In response to a shift of the LD cycle, the light-responsive region of the rat SCN has typically fully shifted or overshifted after a single day (Nagano et al., 2003; Nakamura et al., 2005). So why do the effects of jet lag often persist for many days? Part of the answer is that the peripheral components (oscillatory tissues) of the system adjust at different rates. For example, the rhythms in rats of internal organs such as liver and lung reentrain at rates much slower than that of the SCN (Pittendrigh, 1993; Yamazaki et al., 2000). Temporary desynchrony of the system, called "transient internal dissociation" by Aschoff (1978), can result from the different rates of reentrainment of the individual components, but our modeling reveals that there is another major cause of desynchrony, as we will see. The symptoms of jet lag may partly be due to this transient desynchrony, triggered by abrupt shifts of the LD cycle that cause large changes in the relative phases of the components of the circadian system as they scramble to entrain to the new schedule.

The goal of our circadian system model is to predict the effects of abrupt shifts on a multioscillatory circadian system. Much mathematical modeling has been done of the intracellular circadian rhythms generated by expression of clock genes (e.g., Forger and Peskin, 2003; Leloup and Goldbeter, 2004) and of synchronization of networks of cellular oscillators within a single tissue like the SCN (e.g., Antle et al., 2003; Gonze et al., 2005). However, little modeling has been done to address the coordination of rhythmic tissues and the master pacemaker. Kronauer and colleagues have developed successful models describing the interaction of observable rhythms, such as temperature and sleep-wake cycles in humans (e.g., Kronauer et al., 1982; Gander et al., 1985; Strogatz, 1987; Jewett and Kronauer, 1999). These models couple 2 oscillators to model the output of complex physiological processes and multiple tissues. In contrast, the model developed in the present article treats the coordination of multiple, more basic circadian units of individual oscillatory tissues with a hierarchical, or multistage, organization, based on experimental data for nocturnal rodents such as rats.

MATERIALS AND METHODS

To develop our multistage system model, we first observe that the SCN itself exhibits regions of differing rhythmicity (Yan et al., 1999; Reddy et al., 2002; Nagano et al., 2003; de la Iglesia et al., 2004; Nakamura et al., 2005; Albus et al., 2005), and so it is appropriate to model the SCN as having multiple components. The organization of these compartments is species dependent, so we must make a specific choice, and we focus on the rat. In rats, the ventrolateral region (VLSCN) is light responsive and adjusts very rapidly to shifts of the LD cycle. In contrast, the dorsomedial region (DMSCN) is not directly light responsive, seems to have a shorter period than the VLSCN (Nakamura et al., 2005), and can take up to 2 weeks to adjust to shifts (Nagano et al., 2003). The strong correlation between the phase of the DMSCN and the locomotor activity pattern of rats observed by Nagano et al. (2003) suggests that the DMSCN may play a role as an intermediate component between the VLSCN and at least some peripheral components of the circadian system. Based on this physiological information, our system model (Fig. 1) starts with the LD cycle that entrains a pacemaker oscillator representing the VLSCN (with period 24.3 h, typical of rats), which in turn entrains an intermediate oscillator representing the DMSCN (with period 23.7 h). Peripheral components of our system, representing various oscillatory tissues throughout the body, entrain to the intermediate component.

Our modeling objective is to explore *qualitatively* what dynamics might be expected of a circadian system, and we do not claim to any quantitative accuracy in the sense of curve-fitting experimental data. We choose model parameters to match known dynamic properties such as the free-running period, phase difference from the master pacemaker, and transient dynamics of phase shifting for a 6-h advance and a 10-h delay, based on experimental results for rats. We then use the model to predict what types of transient dynamics can arise in such a system following various shifts of the LD cycle. These predictions are not directly applicable to other species such as humans, but they are certainly suggestive and indicate possible directions for further investigation.

Model for an Oscillatory Component of the Multistage System

Since the purpose of our modeling is to study the dynamics of a system of oscillators, we wish each

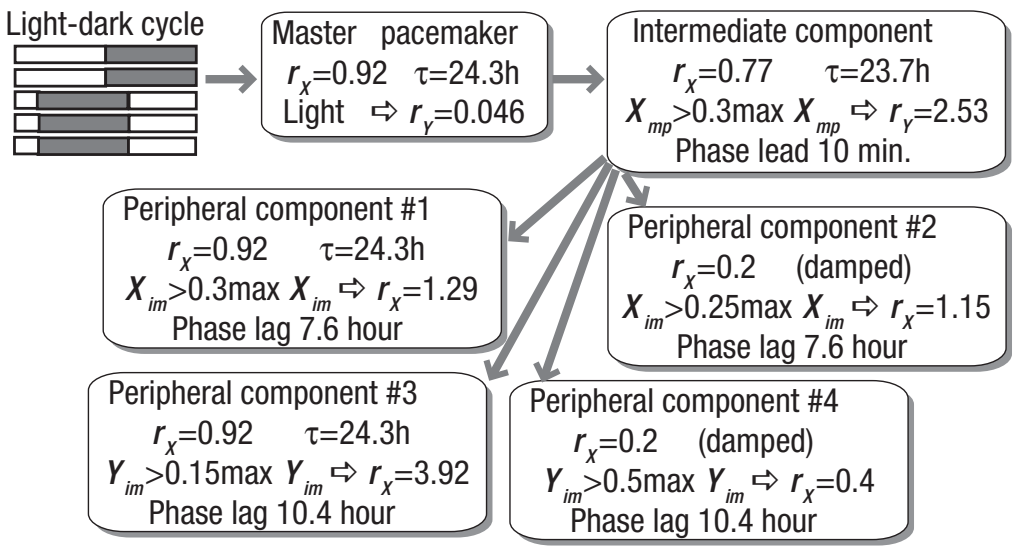

Figure 1. Model of a multistage circadian system. The parameter values and couplings were chosen based on experimental data for nocturnal rodents such as rats. All components have parameter values $r_Y = 0.92$ and $q_X = q_Y = 0.21$, except as otherwise indicated. The phase is relative to the master pacemaker. The physiological connections between components of the circadian system are not yet clear (some are likely neuronal while others are humoral; see Reppert and Weaver, 2002, for a review), and so we use a simple threshold coupling to explore possible dynamics. The threshold is proportional to the maximum value of the indicated variable under entrained conditions (12 h light, 12 h dark), where the subscript *mp* refers to the master pacemaker and *im* refers to the intermediate component.

component oscillator to be relatively simple while producing the following essential circadian dynamics:

1. In the absence of external inputs, the oscillator should "free-run," producing autonomous self-sustained oscillations with an approximately 24-h period.
2. The oscillator should entrain to external cycles with periods near 24 h, for example, the diurnal LD cycle as the Earth rotates.
3. The oscillator should produce an appropriate PRC for the species being modeled.
4. When entrained to an LD cycle, the oscillator should shift in response to advances and delays with transients, as seen in "jet lag" experiments.

For this initial modeling effort, we choose an oscillator model that produces limit cycles in the plane, with the awareness that restricting the oscillator to 2 equations in 2 variables necessitates that most details of the transcriptional-translational feedback loop must be neglected. Therefore, rather than attempting to develop a model tied to biochemical details that we cannot hope to capture in just 2 equations, we seek a "black box" that produces the proper circadian dynamics. After considering various choices, we chose a model developed by Scheper et al. (1999), originally intended to model the interaction of a generic mRNA and protein, although we do *not* interpret the model in this (outdated) way. We use this model because it satisfies the 4 circadian dynamics criteria. This is a very important point: the dynamics of our multistage circadian system will depend on the dynamics of the components, and so these components must exhibit appropriate circadian dynamics. Our use of the Scheper model should be taken in a similar spirit to the way in which, for example, van der Pol oscillators may be successfully used to represent a circadian oscillator without explicit biochemical interpretation.

Each component of our system is modeled using the following system of 2 equations in state variables X and Y:

$$\frac{dX}{dt} = \frac{r_X}{(1 + Y^2)} - q_X X,$$

$$\frac{dY}{dt} = r_Y X^3(t - t_{\text{lag}}) - q_Y Y.$$

Observe the discrete time lag t_{lag} (set to 4 h), which implies that one cannot define isochrons and that the model has dimensionality greater than 2. Besides this time lag, there are 4 parameters for each circadian component, plus coupling parameters when we compose the system, leading to a large set of parameters to determine. We do not attempt to fit to data beyond

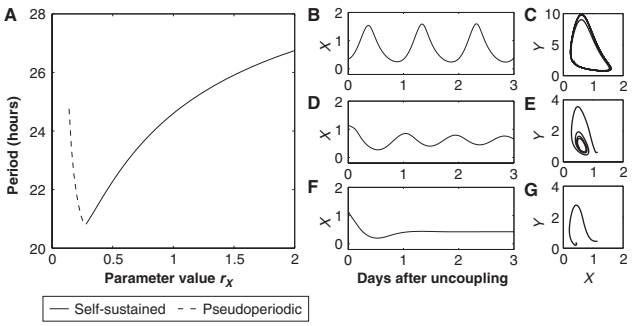

Figure 2. Types of equilibria in the *XY*-plane. (A) Free-running periods in constant darkness versus the parameter r_X (with $r_Y = 0.92$ and $q_X = q_Y = 0.21$). If $r_X > 0.28$, then the model produces self-sustaining oscillations. If $0.14 < r_X < 0.28$, then the solution converges to a stable equilibrium point in a pseudoperiodic manner with a characteristic "period" of spiraling, independent of the period of initial entrainment (see text for further discussion). In simulations with $r_X < 0.14$, the model appears overdamped, converging directly to the equilibrium point. (B) Oscillation of *X* over 3 days for a self-sustaining oscillator with $r_X = 0.77$, yielding a free-running period of 24.3 h. (C) After disconnection from the system, the solution for $r_X = 0.77$ rapidly converges to the free-running limit cycle in the phase plane. (D) Oscillation of *X* over 3 days for a damped component with $r_X = 0.26$. (E) After disconnection from the system, the solution for $r_X = 0.26$ shows pseudoperiodic spiraling with "pseudoperiod" 20.9 h (time between peak *X*) in the phase plane. (F) Oscillation of *X* over 3 days for a damped component with $r_X = 0.1$. (G) The solution for $r_X = 0.1$ goes rapidly to the equilibrium point after disconnection from the system.

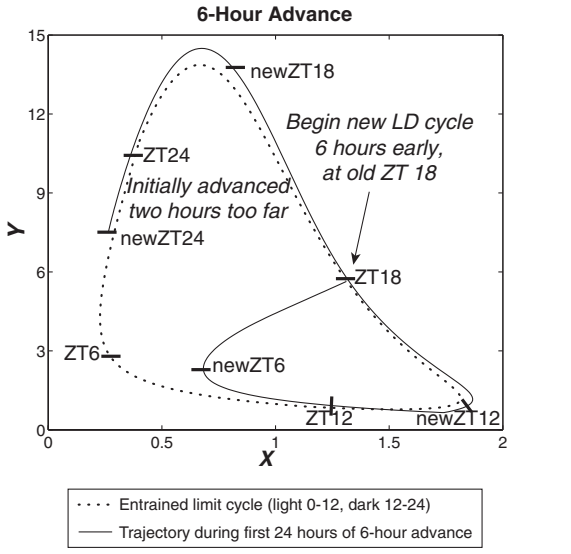

Figure 3. Transient response to a 6-h advance. After a short night of just 6 h darkness, the solution trajectory for the master pacemaker takes a "shortcut" inside the limit cycle, resulting in an immediate 8-h advance of the oscillator's phase (see Fig. 5A for subsequent transients). Zeitgeber times for the original entrained LD cycle are marked "ZT," while times for the advanced LD cycle are marked "newZT."

matching dynamic properties as mentioned above, and we assume similar values across the system for t_{lag}, r_Y, q_X, and q_Y.

This model satisfies our 4 criteria. It exhibits appropriate free-running oscillations with periods near 24 h (depending on parameter values; see Fig. 2A). We achieve entrainment to LD cycles by reducing the production rate r_Y in response to light, which should not be interpreted physiologically,[2] as *X* and *Y* do *not* describe the expression of a gene and should be thought of simply as state variables that track the phase of the oscillator. This choice of light response results in high levels of *X* around ZT 12-19 and peak levels of *Y* around ZT 19 to ZT 1, and it also produces transients of phase shifting in jet lag simulations similar to those observed by Nagano et al. (2003), particularly the initial 2-h overshoot by the VLSCN following a 6-h advance or 10-h delay of the LD cycle (see Figs. 3 and 4). For a typical PRC, see Figure 6B of Scheper et al. (1999).

We would like to point out that the constant response to light in this model is based on the rat SCN and so leads to a strong immediate response that is typical of nocturnal rodents, as well as a weak (type 1) PRC typical of a rat (Honma et al., 1978). This response is qualitatively different from that of humans, who are responsive to light throughout the day (Jewett et al., 1997) and can exhibit strong (type 0) PRCs (Duffy and Wright, 2005). There is also the possibility that peripheral components can exhibit type 0 PRCs (Nagoshi et al., 2004), which is not addressed in our current modeling. The model's light response does not reflect the effect of different light intensities or the reduced responsiveness seen in experiments with multiple light pulses on nocturnal rodents (Khammanivong and Nelson, 2000). From a simulation point of view, this model with constant light response works well for both short light pulses and 12:12 LD cycles, but lengthening the interval of light in the LD cycle can lead to

2. In particular, one should *not* try to connect *X* and *Y* to *Period* gene expression; the state variables *X* and *Y* can be roughly interpreted as describing *Bmal1* expression, with light response interpreted as the decrease in BMAL1-CLOCK activity that is a consequence of strong induction of *Period* genes. However, we do not assign such an interpretation and maintain that it is best to treat the model of *X* and *Y* as a "black box" whose output satisfies our 4 criteria for circadian dynamics.

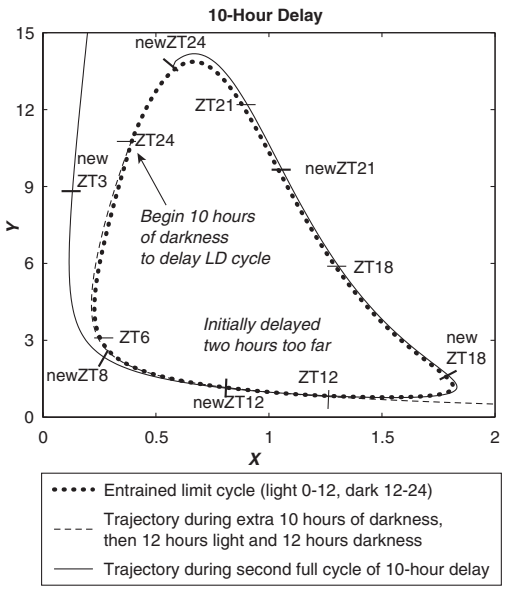

Figure 4. Transient response to a 10-h delay. The 10-h delay of the LD cycle is accomplished by lengthening the night, resulting in an initial large-amplitude transient that quickly settles to the limit cycle but with an initial phase shift of about 12 h (2-h overshoot). Zeitgeber times for the original entrained LD cycle are marked "ZT," while times for the advanced LD cycle are marked "newZT."

period doubling (alternating amplitudes in the daily oscillations) or arrhythmicity. Future modeling beyond this initial work will include greater physiological detail to take into account duration effects and important interspecies differences in light response, particularly the differences between humans and nocturnal rodents.

One advantage of the Scheper model is that it provides a means of modeling both self-sustaining and damped oscillators, as demonstrated by Figure 2A. The idea of "pseudoperiodic" oscillations deserves some explanation. For a damped Scheper oscillator corresponding to a stable spiral point, as in Figure 2D,E, the X state variable repeatedly attains a local maximum value at regular time intervals (if the trajectory begins near the equilibrium point and the system is otherwise unperturbed). The length of this time interval between local maxima is what we call the "pseudoperiod." As a more familiar example, consider the case of an underdamped spring or linearized pendulum with friction—for example, $x'' + 4x' + 13x = 0$, which (once set in motion) will swing back and forth in a regular fashion with general solution $x(t) = Ae^{-2t}\cos(3t + \phi)$. The amplitude Ae^{-2t} of the solution decays to 0 over time, yet $x(t)$ has regularly spaced local maxima yielding a pseudoperiod of $2\pi/3$. This is not true periodic behavior since the amplitude is decaying, but one can still define the pseudoperiod in a practical manner as the time between peak values of a state variable such as X if the solution is near a stable spiral equilibrium point.

Multistage System Model

Now we must specify how to connect the component oscillators. The master pacemaker of our multistage system is a light-responsive component representing the VLSCN; this component responds to light by severely reducing the protein production rate r_Y (the default value is valid for darkness). The intermediate component is tentatively identified here as the DMSCN, but other brain areas near the SCN may play a similar role. Its Y-production is stimulated if the master pacemaker's X-abundance rises above a certain threshold. This naturally induces a phase-dependent coupling, with the intermediate component slightly phase-leading the master pacemaker. While this coupling is not physiologically based (there is too little physiological knowledge to derive a coupling), its results are in good agreement with experimental data, in which the phase of the DMSCN appears to be about the same as that of the VLSCN (Nagano et al., 2003) or leading by less than an hour (Nakamura et al., 2005). This coupling also reproduces the observed transient dynamics of the VLSCN and the DMSCN following a 10-h delay or 6-h advance, as reported by Nagano et al. (2003).

Finally, we need to connect the peripheral components to the intermediate component. In experiments, peripheral oscillators typically lag the SCN by 6 to 11 h. For example, Yamazaki et al. (2000) observed that the phase of lung tissue lagged the SCN by about 7 h, while that of the liver lagged by 10 to 11 h. Since it is not clear whether peripheral tissues can self-sustain oscillations, as the SCN clearly does, we include both a self-sustaining and a damped peripheral component to illustrate the multistage dynamics in our model and to test whether these show a difference in entrainment behavior. In our model, if the abundance of X (for components 1 and 2) or Y (for components 3 and 4) in the intermediate component is above a certain threshold, then the r_X parameter of the peripheral component is increased (see Fig. 1 for details). These choices were arbitrarily made so that the peripheral components lag the VLSCN by about 7.6 h for components 1 and 2, and by about 10.4 h for components 3 and 4 (under entrained conditions of 12 h light

Table 1. Reentrainment Times in the Multistage Circadian System

Shift (h)	*Predicted VLSCN (Day)*	*Data VLSCN (Day)*	*Predicted DMSCN (Day)*	*Data DMSCN (Day)*	*Predicted #1 (Day)*	*Predicted #2 (Day)*	*Predicted #3 (Day)*	*Predicted #4 (Day)*
–12	2		8		10	10	10	8
–10	4 (O)	4 (O)	7	5-7	9	9	9	6
–8	4 (O)		6		8	8	8	6
–6	4 (O)		4		7	7	6	5
–4	2		7		8	7	7	7
–2	2		6		7	6	6	6
2	3		5		6	6	5	4
4	3		7		8 (DA)	8	8	6
5	4		10 (DA)		12 (DA)	11	12 (DA)	10 (DA)
6	4 (O)	4-5 (O)	13 (DA)	9-13 (DA)	13 (D)	14 (DA)	13 (DAD)	13 (DA)
7	4 (O)		12 (D)		13 (D)	13 (D)	13 (D)	12 (D)
8	4 (O)		10 (D)		12 (D)	12 (D)	12 (D)	10 (D)
9	5 (D)		8 (D)		11 (D)	10 (D)	11 (D)	8 (D)
10	5 (D)		8 (D)		10 (D)	10 (D)	10 (D)	8 (D)
11	3 (D)		8 (D)		10 (D)	10 (D)	10 (D)	8 (D)

NOTE: The table lists the day on which reentrainment is completed to within 1/2 hour for indicated delays and advances of the LD cycle. The shift of the LD cycle occurs on day 1 by delaying the start of day for delays and shortening the night for advances. We give both our model's predictions for each component of the multistage system and experimental data (Nagano et al., 2003), which gives the level of *rPer1* mRNA every 2 h for a 10-h delay and a 6-h advance. Phase in our model is based on peak of *X*, while phase from the data corresponds to peak of measured *rPer1* abundance. Negative numbers denote delays, while positive numbers denote advances. Shifts marked (D) are advances accomplished by delaying transients (e.g., adjusting to a 9-h advance by delaying 15 h), while (DA) indicates that the oscillator's phase on day 2 was delayed, but the shift was eventually accomplished as an advance. The notation (DAD) means that the phase showed delaying transients on the first 3 days, advancing transient on the 4th day, and then accomplished the shift as a delay. The notation (O) indicates that the phase on day 2 overshot the desired phase by 1 to 3 h. Nagano et al. (2003) observed that the rat VLSCN overshoots the desired phase by 2 h on day 2 of a 10-h delay, while for a 6-h advance, the overshoot of 2 h appears on day 2 and persists for several days. VLSCN = ventrolateral region in the SCN; DMSCN = dorsomedial region in the SCN.

and 12 h darkness). These peripheral components are merely examples, not intended to truly represent a particular tissue such as the liver or lung.

While the coupling in our multistage model is heuristic, it should nevertheless reveal the gross system dynamics that emerge from entrainment and jet lag conditions for a hierarchy of circadian oscillators. Other choices of coupling the oscillators lead to different phase differences between the master pacemaker and the other components but qualitatively similar results in transient dynamics.

We used MATLAB (The MathWorks, Natick, MA) to generate simulations, with a maximum step size of 0.05 h in the adaptive dde23 solver.

RESULTS

In simulations of our system model (see Table 1), the master pacemaker shifts rapidly for both advances and delays, with reentrainment time longer for larger shifts due to the phase overshooting the desired phase on day 2 (requiring 1-3 further days for the phase to relax back to the desired position), with the exception that 12-h shifts are rapidly accomplished since there is no overshoot. The other components of the system shift more gradually. In particular, advances of 5 and 6 h are slow due to the initial delaying of some components before proceeding to advance. Such initial delaying has been observed in rat lung tissue by Yamazaki et al. (2000).

The desynchrony of the system can be extreme following a 6-h advance (Fig. 5A). The master pacemaker advances rapidly, overshooting the desired phase for several days; the intermediate and damped peripheral components initially delay but then advance and overshoot the desired phase; and the self-sustaining peripheral oscillator steadily delays to accomplish the 6-h advance as an 18-h delay. On day 6, the intermediate component appears to have moved to the desired phase, but on day 7, the phase has again advanced several hours and takes a further week to settle to the proper phase.

Antidromic reentrainment[3] has a significant impact on the system dynamics. For delays and short advances of the LD cycle (for which the system exhibits

3. Antidromic reentrainment refers to a slave shifting in the opposite direction as the master; the typical case in our context occurs when the phase of the master pacemaker *advances* by 6 h but the intermediate component's phase shifts by *delaying* a few hours each day. Orthodromic reentrainment refers to both oscillators shifting in the same direction.

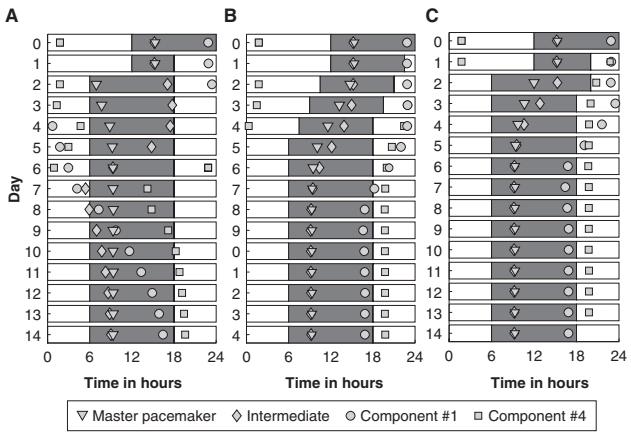

Figure 5. Jet lag simulations. The system is first entrained to the LD cycle shown on day 0 for 30 days, after which the LD cycle is shifted starting on day 1, as indicated by the bars. White bars correspond to light, while black bars correspond to darkness. We use the peak time of *X* to mark the phase of each oscillator. (A) A 6-h advance of the LD cycle, with a night shortened by 6 h, results in transient desynchrony for 2 weeks. (B) A 6-h advance of the LD cycle, with the first 4 days each advanced by 1.5 h, resulting in an orderly but gradual transition. This protocol mimics the entrainment schedule in Revell and Eastman (2005) of advancing a small amount each day. (C) A 6-h advance of the LD cycle similar to that shown in (A), but with an extra 2 h of darkness before dawn on days 1 and 2, results in rapid and orderly reentrainment. The same protocol leads to rapid and orderly reentrainment dynamics for 5- to 8-h advances.

orthodromic reentrainment), it is true that greater shifts generally imply longer reentrainment periods. However, in the simulations shown in Table 1, advances of 5 to 8 h require more reentrainment time than longer advances of 9 to 12 h, due to some (but not all) of the components showing antidromic reentrainment (i.e., reentrainment by partition). This range of difficult advances varies with the coupling strength between the master pacemaker and the intermediate component (and can even be pushed over to a range of delays). For example, if the coupled value of r_Y in the intermediate component is increased, the phase difference from the SCN decreases, as does the switch point between orthodromic and antidromic reentrainment in the intermediate component.

Numerical simulations indicate that for relatively strong coupling strengths, reentrainment switches from orthodromic to antidromic abruptly (as the length of the LD shift is increased) but without lengthening the reentrainment time. Quite a different behavior emerges for weak coupling strengths, in which the reentrainment time grows much longer the closer the shift is to the switch point (typically near a 10-h advance), so that for shifts of the LD cycle near this critical switch point, the component can appear "stuck" for a long time.

DISCUSSION

Properties of a Multistage System

Our modeling reveals some important insights about the stability and flexibility of the multistage circadian system. The master pacemaker adapts swiftly to changes in the LD cycle, providing the system flexibility in adjusting to such changes. The intermediate component provides stability, in the sense that it provides a buffer against small perturbations of the LD cycle. For example, a 4-h delay of the LD cycle for a single cycle (followed by resumption of the original LD cycle) results in a 3.4-h shift of the SCN for a day but at most 1.2 h for the DMSCN and peripheral components over the next few days. Our multistage model also demonstrates that having multiple stages does not mean that reentrainment times will necessarily get progressively longer with each stage beyond the intermediate oscillator. Hence, the intermediate stage oscillator can add stability to the multistage system without costing additional reentrainment time of the peripheral components.

A Slave Can Phase-Lead Its Master

As seen in the simulations of the master pacemaker and the intermediate oscillator, a slave oscillator can phase-lead the master. The fact that one oscillator peaks earlier than another does not directly indicate which one is in fact the pacemaker. The phase difference between a master and a slave oscillator can be set to nearly any desired value (either leading or lagging) through appropriate construction of the coupling mechanism and depends predominantly on which variable (*X* or *Y*) of the master used to entrain the slave and which parameter of the slave is affected (e.g., r_X or r_Y). The phase difference is also affected to a lesser extent by the threshold value and the strength of the coupling. Kronauer et al. (1982) also observed such coupling effects on relative phase in their 2-oscillator model.

Self-Sustaining versus Damped Components

Changing a model parameter, such as a production or degradation rate, affects the free-running period

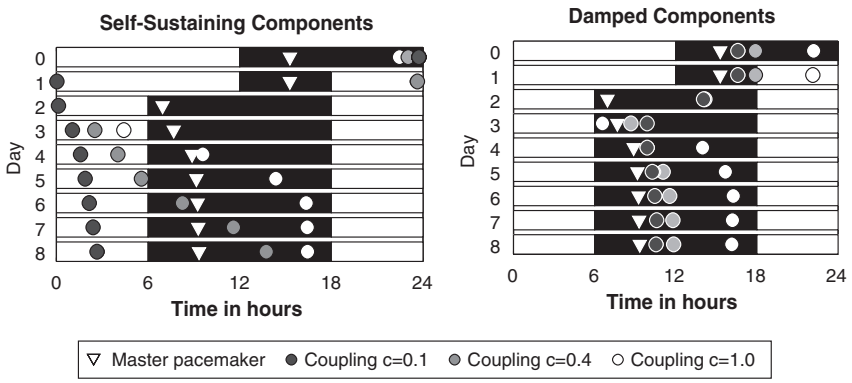

Figure 6. Comparison of self-sustaining and damped components. Simulations of a 6-h advance of the LD cycle show markedly different dynamics for self-sustaining ($r_x = 0.92$) and damped ($r_x = 0.145$) components that are directly connected to the master pacemaker. The free-running period (or pseudoperiod if damped) of each component is 24.3 h. Coupling strength is defined here to be the relative increase in r_x when the master is above threshold. For self-sustaining components, the coupling strength has only a small effect on the relative phase but a large effect on the rate of reentrainment (weaker couplings result in slower reentrainment rates). For damped components, the coupling strength has a large effect on the relative phase. Weak couplings result in the damped component's phase closely following that of the master pacemaker and quickly reentraining following shifts of the LD cycle (rather like a dog heeling its master), while a stronger coupling enables a damped component to act more like a robust oscillator, including more gradual reentrainment behavior. Also note that increasing the coupling strength for a self-sustaining component results in a decreased phase lag but, for a damped component, results in an increased phase lag.

and can even cause a change from self-sustaining to damped oscillations. This fact suggests that in vitro experiments, which are likely to cause changes in the physiological equivalents of these rate parameters, can lead to different free-running behavior than would be seen in vivo for the same tissue. The fairly steady "period" of damped oscillations seen in luminescence experiments could be the result of the in vitro conditions causing a tissue to exhibit damped oscillations with a steady pseudoperiod possibly different from the in vivo period. Yoo et al. (2004) have suggested a similar hypothesis for explaining damping in vitro.

Another possibility is that the damped oscillations observed in vitro could result from a collection of cells that each individually maintain a robust rhythm but drift out of phase with each other, resulting in a decreasing amplitude of the ensemble rhythm. This was suggested by Balsalobre et al. (1998) and was recently observed in fibroblasts by Welsh et al. (2004). The circadian oscillations of cells in peripheral tissues could be weakly coupled, requiring a strong signal from the master pacemaker to synchronize. In contrast, within each compartment of the SCN exhibiting synchronized oscillations, the intercellular coupling might be relatively strong, resulting in the much longer coherence of the ensemble typically observed in vitro.

In our simulations, damped components that receive weak input from the intermediate component tend to exhibit a marked lack of variation in shifting transients, while damped components with stronger input act similarly to robust oscillators (see Fig. 6). Based on our observations from these simulations, we suggest 3 different possibilities to explain the fact that the rate of entrainment differs among both tissues (Yamazaki et al., 2000) and measurable physiological functions (Klein and Wegmann, 1980):

1. Some peripheral tissues might be truly damped, in the sense that individual cells cannot sustain oscillations, and the variation of entrainment rates could be due to different intermediate components (which are each self-sustaining oscillators) connecting the peripheral component to the master pacemaker. The variation of entrainment rates among the intermediaries would be passed down to their slaved components.
2. Some peripheral tissues might be composed of cells that are self-sustaining oscillators but lack sufficient intercellular connections to synchronize in the absence of an external pacemaker. When connected to the pacemaker, the component oscillates robustly enough that its qualitative behavior can be indistinguishable from that expected of self-sustaining oscillators.
3. Some peripheral tissues may be composed of strongly coupled cells that exhibit self-sustaining oscillations, so that the tissue itself is a self-sustaining oscillator. Such components can entrain to the master pacemaker but are not dependent on it for maintaining oscillations. The rate of reentrainment depends on the strength of coupling with the master pacemaker (weak coupling strengths generally correspond to slow reentrainment rates; see Fig. 6). They may exhibit damped oscillations in vitro due to change of environmental conditions.

Implications for Jet Lag Prevention

In our model, advances of 5 to 8 h tend to be harder than other shifts because the intermediate and peripheral components respond by delaying even though the pacemaker oscillator advances immediately. The pacemaker oscillator exhibits a sharp discontinuity of behavior: it advances rapidly in response to advances of less than 8.3 h and otherwise delays rapidly (fully shifted or overshifted on day 2 for the former case, on day 3 for the latter). The intermediate oscillator exhibits the same discontinuity of behavior in response to advances. Due to the overshoot of the master pacemaker's phase for several days following a 6- to 8-h advance, the intermediate oscillator initially perceives an 8- to 10-h advance and so delays to accomplish advances greater than 6.3 h. For advances of 5.0 to 6.3 h, the intermediate oscillator initially delays but then accomplishes the shift by advancing. Hence, the overshoot of the master pacemaker triggers desynchrony by causing reentrainment by partition.

To accomplish the 6-h advance without the desynchrony seen in Figure 5A, we advance dawn on days 1 and 2 by just 4 h, as shown in Figure 5C. This change in the shifting protocol is based on the observation that, in simulations of advances of the LD cycle, a 4-h advance results in the greatest systemwide advance by day 3, giving the system a strong push in the "right direction" and causing all components to smoothly and rapidly advance to the desired phase, even more quickly than the gradual protocol shown in Figure 5B. This procedure can be applied more generally to reduce desynchrony during difficult advances: find the largest advance for which a given system remains orderly during the first few days and then incorporate it into the shifting protocol.

Evidence of circadian components displaying antidromic reentrainment has appeared in human studies, in which body temperature rhythms and urinary excretion of chloride, sodium, calcium, and phosphate adapted to 6- to 9-h advances by delaying rather than advancing in an increasing proportion of human subjects as the shift length increased (Wever, 1970; Klein et al., 1977; Mills et al., 1978; see also Boulos et al., 1995; Revell and Eastman, 2005). In these studies, reentrainment by partition often occurred, with some rhythms exhibiting delaying transients while others exhibit advancing transients, qualitatively similar to the transient desynchrony shown in Figure 5A. These studies, combined with the results of our modeling, suggest that preventative treatments for jet lag should not necessarily be aimed at most rapidly shifting the master pacemaker. A better strategy may be to consider system dynamics, aiming at the largest shift of the master pacemaker that still leads to coordinated shifting of the system as a whole. While our model is based on circadian dynamics of nocturnal rodents, the general principle is likely applicable more widely and merits further investigation with species-appropriate models.

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