Light-Induced Resetting of the Circadian Pacemaker: Quantitative Analysis of Transient versus Steady-State Phase Shifts

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Abstract The suprachiasmatic nuclei of the hypothalamus contain the major circadian pacemaker in mammals, driving circadian rhythms in behavioral and physiological functions. This circadian pacemaker's responsiveness to light allows synchronization to the light-dark cycle. Phase shifting by light often involves several transient cycles in which the behavioral activity rhythm gradually shifts to its steady-state position. In this article, the authors investigate in Syrian hamsters whether a phase-advancing light pulse results in immediate shifts of the PRC at the next circadian cycle. In a first series of experiments, the authors aimed a light pulse at CT 19 to induce a phase advance. It appeared that the steady-state phase advances were highly correlated with activity onset in the first and second transient cycle. This enabled them to make a reliable estimate of the steady-state phase shift induced by a phase-advancing light pulse on the basis of activity onset in the first transient cycle. In the next series of experiments, they presented a light pulse at CT 19, which was followed by a second light pulse aimed at the delay zone of the PRC on the next circadian cycle. The immediate and steady-state phase delays induced by the second light pulse were compared with data from a third experiment in which animals received a phase-delaying light pulse only. The authors observed that the waveform of the phase-delay part of the PRC (CT 12-16) obtained in Experiment 2 was virtually identical to the phase-delay part of the PRC for a single light pulse (obtained in Experiment 3). This finding allowed for a quantitative assessment of the data. The analysis indicates that the delay part of the PRC—between CT 12 and CT 16—is rapidly reset following a light pulse at CT 19. These findings complement earlier findings in the hamster showing that after a light pulse at CT 19, the phase-advancing part of the PRC is immediately shifted. Together, the data indicate that the basis for phase advancing involves rapid resetting of both advance and delay components of the PRC.

Key words circadian rhythms, suprachiasmatic nucleus, entrainment, phase shift, transients, phase response curve, evening/morning oscillators

An endogenous circadian pacemaker in the suprachiasmatic nuclei (SCN) determines the timing of many behavioral and physiological functions (Meijer and Rietveld, 1989). For proper timing, this

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pacemaker is responsive to the environmental light-dark cycle. Light presented during the beginning of the subjective dark period induces phase delays of the circadian activity rhythm, whereas light presented toward the end of the subjective dark period produces phase advances. The effects of light on the circadian activity rhythm can be described by a phase response curve (PRC). In a PRC, the magnitude and direction of phase shifts in activity onset are plotted as a function of the circadian time of pulse application (Daan and Pittendrigh, 1976).

After a light pulse, a phase shift is often not completed within the first circadian cycle but instead grows over the course of several days. Such circadian cycles are called *transient cycles*. They are most pronounced following phase-advancing light pulses, but they occur after delaying pulses as well (Pittendrigh et al., 1958). It is not clear whether activity onset during the transient cycles reflects the true position of the underlying pacemaker. One possibility is that the pacemaker itself requires several days to complete the phase shift. Alternatively, the pacemaker may shift immediately to its final position but the activity rhythm requires several days to become fully synchronized with the new phase of the pacemaker.

On the molecular level, it has been shown that the expression of the mammalian homologs of the insect *period* gene, *mper1* and *mper2*, peak 1 h and 2 h after application of a light pulse, respectively (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997). It is clear that resetting mechanisms are induced very quickly and increases in the putative protein products of *mper* may be the cause of resetting to a new phase. The finding that inhibition of *mper1* expression blocks the phase shifts induced by light and glutamate pulses supports the notion that *mper* is required for phase shifting by light (Akiyama et al., 1999). It appears therefore that resetting of the clock is established within a few hours. To understand the discrepancy between the molecular biology and the behavior of the overt rhythm, it is important to get insight into the position of the pacemaker during the transient cycles in vivo. For this purpose, it is necessary to determine the position of the PRC during the transient cycle, assuming that the position of the PRC provides a true reflection of the phase of the pacemaker.

One way to investigate the position of the PRC is by applying a second light pulse during the first transient cycle after a light pulse and to investigate the phase-shifting effect of the two light pulses on the final phase of the activity rhythm. Two-pulse experiments have been performed in invertebrates such as *Drosophila* (Pittendrigh, 1979) and *Neurospora* (Crosthwaite et al., 1995), in mice (Sharma and Chandrashekaran, 2000), and in Syrian hamsters (Elliot and Pittendrigh 1996; Best et al., 1999). In the hamster, it has been established that the phase-advance part of the PRC shifts within a few hours after a light pulse at CT 18 by applying a second light pulse 1 to 2 h after the first pulse (Best et al., 1999). It was also shown that the phase-delay part shifts within a few hours after a light pulse at CT 13 by applying a second pulse 1 to 2 h after this pulse (Best et al., 1999). However, it has not been investigated whether the phase-delay part of the PRC shifts immediately after a phase-advancing light pulse. This matter is of great importance in view of recent findings, indicating that the pacemaker is composed of distinct genetic components that exhibit different responsiveness to phase-advancing and phase-delaying light pulses (see Daan et al., 2001).

We investigated the responsiveness of the phase-delay part of the PRC during a transient cycle that was induced by a phase-advancing pulse. To this purpose, we applied a second light pulse shortly after activity onset at the first transient cycle. By comparing the effect of a single light pulse with the effect of the double light pulse, we could investigate the phase-shifting effect of the second light pulse. From the magnitude of the phase shift, it can be estimated what the position of the circadian clock was at the time point of application of the second light pulse. To optimize this estimate, we determined the relation between activity onset during the first transient cycles and the final steady-state phase after application of a single phase-advancing light pulse. This enabled us to make a reliable estimate of the steady-state phase shift after the first activity onset.

METHODS

This study was performed on 60 male Syrian hamsters (*Mesocricetus auratus*, Harlan/CPB, Zeist, the Netherlands) aged 2 months at the start of the experiment. The animals were individually housed in cages (36.5 × 25.0 × 16.0 cm) with a running wheel (diameter 26 cm) and were kept in a sound-attenuating, ventilated room at a temperature of 23 °C. Food (Hope farms B.V. the Netherlands) and water were continuously available. Running-wheel activity was recorded per minute to determine the animals' circadian activity rhythm. In all the experiments, light pulses (15

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min) were presented to groups of animals, and the protocol was repeated three times. Positioning of the light sources on the wall behind every single cage ensured similar light levels (100-120 lux) for all animals. During presentation of the light pulses, the animals remained in their home cages. All animals received all three treatments, and treatments were presented in a randomized order. At least 1 month elapsed between application of subsequent light pulses. Data were excluded from the analysis when the actogram of running-wheel activity did not allow for unambiguous determination of activity onsets.

Experiment 1

The animals were entrained to LD 14:10 for at least 7 days before they were released into constant darkness (DD). After 7 days in DD (Day 0), the animals received a light pulse between CT 17.77 and CT 21.90. After the light pulse, the animals were kept in DD for 14 days. Lines were eye-fitted through activity onsets before and after the light pulse. The first transient cycles after the light pulse were excluded from the fit. Steady-state phase shifts were determined by measuring the difference between the fitted lines extrapolated to the first cycle after the light pulse (Day 1, Fig. 1A). In addition, the immediate phase shift on the first (Day 1) and second circadian cycle (Day 2) after the light pulse were determined. The immediate phase shift was defined as the difference between the time of observed activity onset and the time as predicted by the line through activity onsets before the light pulse.

A strong correlation between the immediate phase shift on Day 1 and the steady-state phase shift was found. Moreover, we found a strong correlation between the immediate shift on Days 1 and 2 (for further details, see Results and Fig. 3 B,C). These correlations were used for the analysis of Experiment 2.

Experiment 2

A light pulse was applied between CT 17.75 and CT 21.71 according to the protocol as in Experiment 1. In this experiment, the light pulse was followed by a second light pulse given 0.01 to 4.31 h after activity onset on the first transient cycle (Day 1, Fig. 1B). After the second light pulse, the animals were kept in DD for 14 days. The immediate phase shift induced after the first and second light pulses and the steady-state phase shift induced by the two light pulses were determined.

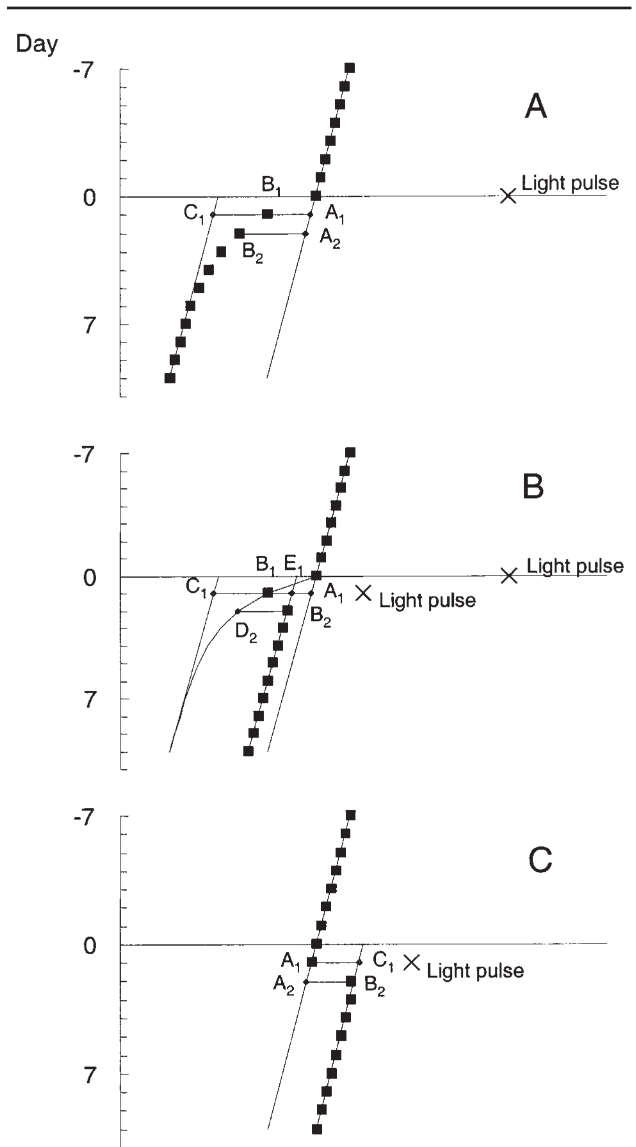

Figure 1. Experimental protocol (see Methods for explanation of the protocol). A. Experiment 1: A light pulse was applied between CT 17.77 and CT 21.90 on Day 0. The steady-state phase shift ($\Delta\phi_{st}$, $A_1 - C_1$), and the immediate phase shift on Day 1 ($\Delta\phi_{im(1)}$, $A_1 - B_1$) and Day 2 ($\Delta\phi_{im(2)}$, $A_2 - B_2$) were measured. B. Experiment 2: A light pulse was applied between CT 17.75 and CT 21.71 on Day 0 and a second pulse was given 0.01 to 4.31 h after activity onset on Day 1. Steady-state phase shifts ($\Delta\phi_{st}$, $E_1 - C_1$) and immediate phase shifts ($\Delta\phi_{im}$, $D_2 - B_2$) induced by the second light pulse were measured. C. Experiment 3: A light pulse was applied between CT 10.80 and CT 15.43 on Day 1. Steady-state phase shifts ($\Delta\phi_{st}$, $C_1 - A_1$) and immediate phase shifts ($\Delta\phi_{im}$, $B_2 - A_2$) were measured.

We considered that the steady-state phase shift induced by the second light pulse is equal to the difference between the steady-state phase shift induced by the first light pulse and the steady-state phase shift induced by the two pulses together. To estimate the steady-state phase shift induced by the first light

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pulse, the strong correlation between the immediate phase shift and the steady-state phase shift obtained in Experiment 1 was used. In other words, the immediate phase shift on Day 1 was determined and the steady-state phase shift ($A_1 - C_1$) for that particular animal was estimated on the basis of the regression line obtained in Experiment 1 (Fig. 3B). To determine the steady-state phase shift induced by the two light pulses, the difference between the steady-state activity onset lines before and after the two light pulses was calculated ($A_1 - E_1$). The steady-state phase shift induced by the second light pulse only is then $(A_1 - E_1) - (A_1 - C_1) = C_1 - E_1$.

Similarly, the immediate phase shift induced by the second light pulse was estimated by measuring the difference between the time of observed activity onset on Day 2 (B_2) and the time of activity onset on Day 2 when the second light pulse would not have been given (D_2). This latter value was predicted from the strong correlation between immediate shifts on Day 1 and Day 2 obtained in Experiment 1.

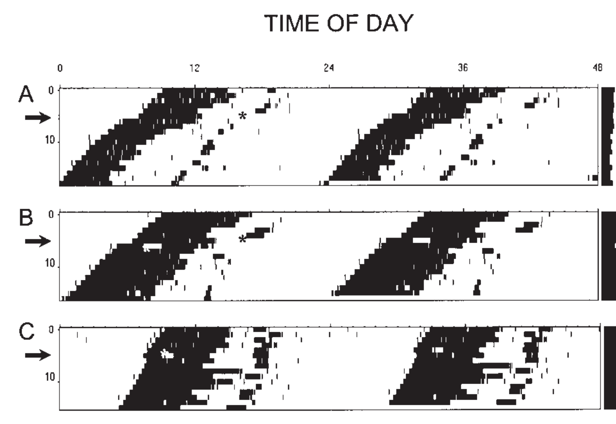

Figure 2. Typical examples of activity records of all three experimental conditions in the same animal. The activity record is double-plotted to enable visualization of the activity rhythms. Arrows and stars indicate day and time of light pulse application. Panel A shows a phase advance after application of a light pulse at CT 21. Panel B shows a phase shift after application of a light pulse CT 21.5 followed by a light pulse applied 2.75 h after activity onset on the next circadian cycle. Panel C shows a phase delay after application of a light pulse at CT 14.

Experiment 3

A light pulse was presented according to the protocol as in Experiment 1. The light pulse was applied between CT 10.80 and 15.43 on Day 1. After the light pulse, the animals were kept in DD for 14 days. The steady-state phase shift on the day of the light pulse (Day 1) was determined by measuring the difference between the fitted lines before and after the light pulse (Fig. 1C). The immediate phase shift on the first cycle after the light pulse (Day 2) was measured as the difference between the time of observed activity onset and the time predicted by the line through activity onsets preceding the light pulse. ANOVA and post hoc *t* tests served to compare Experiment 2 and Experiment 3.

RESULTS

Experiment 1

In Experiment 1, 93 phase advances were analyzed (Fig. 2A). These light pulses were presented between CT 17.77 and CT 21.90 (mean = 19.07). The effect of the light pulse on the steady-state phase shift, $\Delta\phi_{st}$, and on the immediate phase shift on the first cycle after the light pulse, $\Delta\phi_{im(1)}$, are summarized in Figure 3A. The magnitudes of the steady-state phase shift and the immediate phase shift were 1.42 ± 0.44 h and 0.64 ± 0.24 h, respectively (mean ± SD). Although the same animals received light pulses several times, the difference between interindividual and intraindividual variations was not significant for the magnitudes of both steady-state and immediate phase shifts. Therefore, phase shifts obtained from the same animal were treated as independent values in all of the experiments.

The results of this experiment are important for the protocol of the next experiment. As is evident from Figure 3A, a rather large range of phase shifts can be obtained at each circadian time. However, when plotting the steady-state phase shift, $\Delta\phi_{st}$, as a function of the magnitude of the immediate shift on Day 1, $\Delta\phi_{im(1)}$ (Fig. 3B), a strong relation is observed between immediate and steady-state phase shift, which can be described as follows:

$$\Delta\phi_{st} = 1.462 \times \Delta\phi_{im(1)} + 0.482 \ (r = 0.79, p < 1*10^{-20}).$$

This regression line was used to predict for each animal the steady-state phase shift from the immediate shift on Day 1 in Experiment 2.

Moreover, a strong correlation between the magnitude of the immediate shift on Day 1, $\Delta\phi_{im(1)}$, and the immediate shift on Day 2, $\Delta\phi_{im(2)}$, was found (Fig. 3C). This correlation ($r = 0.89$, $p < 1*10^{-20}$) can be described by the following function:

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$$\Delta\phi_{im(2)} = 1.325 \times \Delta\phi_{im(1)} + 0.150.$$

This regression line was used in Experiment 2 to predict for each animal the time of activity onset on Day 2 in the case that only the first light pulse would have been applied and to measure the difference with the activity onset on Day 2.

Experiment 2

In the double pulse experiment, 85 activity records were obtained that allowed for an unambiguous analysis (Fig. 2B). The first light pulse fell between CT 17.75 and CT 21.71 (mean = 19.48), inducing a phase advance on Day 1 in all cases (range from 0.24 to 1.26 h; mean = 0.76 h). The second pulse was given 0.01 to 4.31 h (mean = 1.61 h) after activity onset on Day 1. Those cases where the second light pulse fell before activity onset were excluded from the analysis since no estimate of activity onset on Day 1 could be made. In most cases, the second light pulse induced a phase delay but a few advances were also observed.

Experiment 3

Eighty-five clear phase-shifts were obtained from light pulses that fell between CT 10.80 and CT 15.43 (mean = 12.95) and were used to describe the phase-delay part of the PRC (Fig. 2C). The maximum delay was obtained around CT 13 (Fig. 4). Mean hourly values for the immediate and steady-state phase shift were calculated between CT 11 and CT 16 and were compared with the data from Experiment 2.

Comparison of Experiments 2 and 3

In Experiment 2, the second light pulse was given at the beginning of the subjective night. The phase shift induced by this second light pulse was compared with the shift induced by a single light pulse applied at comparable phases (CT) in Experiment 3. The circadian time of application of the second light pulse is unknown. However, there are two predictions for the circadian time of the second light pulse. (1) If the overt rhythm is the manifestation of the underlying oscillator during transient cycles, the time of the activity onset on Day 1 is equal to CT 12. (2) If the steady-state phase shift reflects the real position of the oscillator, the extrapolated steady-state phase shift on Day 1 is equal to CT 12.

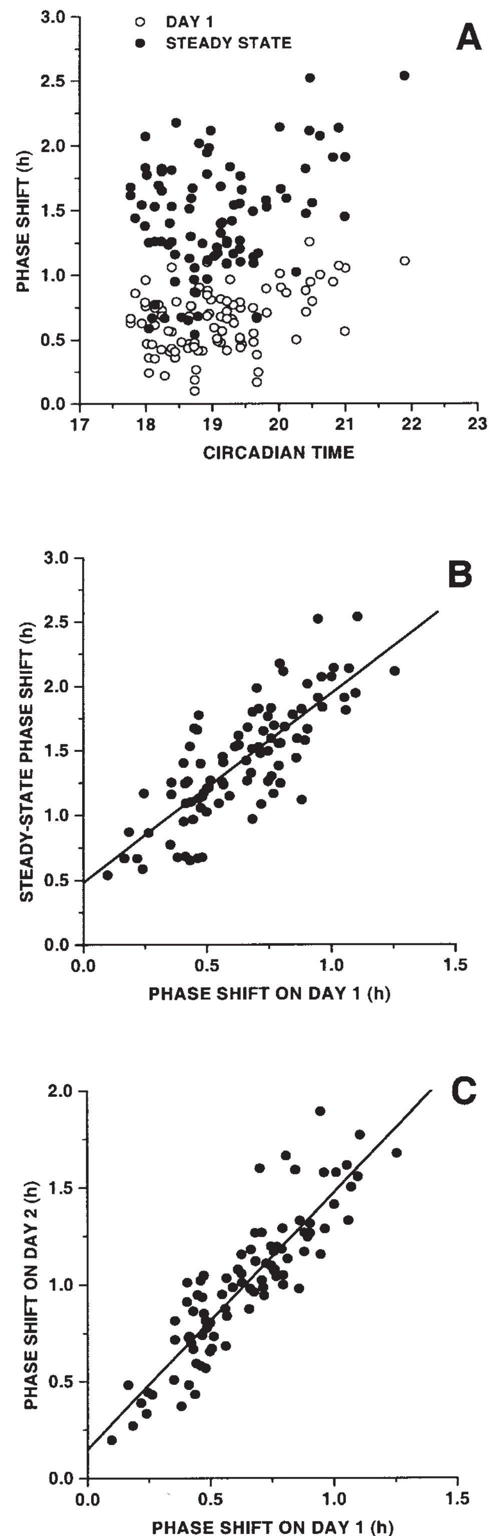

Figure 3. Immediate and steady-state phase shifts induced by a phase-advancing light pulse. A. The phase shifts are plotted as a function of the time of light pulse application. Dots indicate steady-state phase shifts, circles immediate shifts. B. The steady-state shift is plotted as a function of the immediate shift on Day 1. C. The immediate phase shift on Day 2 is plotted as a function of the immediate shift on Day 1.

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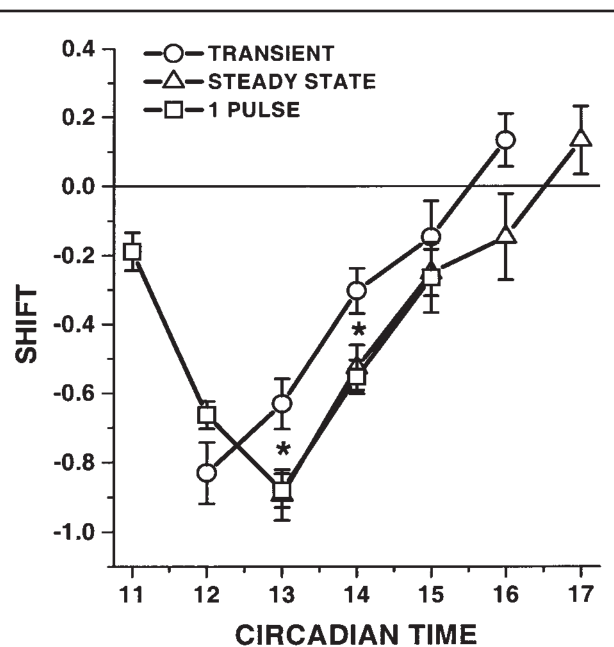

Figure 4. Comparison of phase shifts induced by the single delaying light pulse and those induced by the second of the double light pulse. Hourly values (± SEM) of steady-state phase shifts induced by the second light pulse are plotted against CT of the second light pulse in "steady-state phase" (triangles) or that in "transient phase" (circles) together with the phase shift induced by the single light pulse (squares). Asterisks indicate significant differences between the conditions ($p < 0.05$, two-tailed t test after significant ANOVA for factor "condition" over CT 13 to CT 15).

The two possible phase delays obtained in Experiment 2 with the second light pulse were calculated and compared with the data obtained in Experiment 3 (Fig. 4). The analysis indicates that the steady-state phase shift in Experiment 2 did not differ significantly from the PRC obtained in Experiment 3 when supposing that the pacemaker shifted to its steady-state position within one circadian cycle (Fig. 4, Prediction 2). In contrast, significant differences were obtained at CT 13 and CT 14 between the steady-state phase shifts in Experiment 2 and Experiment 3 when supposing that the first transient indicates the phase of the pacemaker (Prediction 1). Also, the two predicted PRCs differed significantly at CT 13 and CT 14 (Fig. 4).

DISCUSSION

The question posed in this article is whether transient cycles accompanying a light-induced phase advance reflect the circadian time of the pacemaker or whether the pacemaker shifts immediately to its steady-state position. We specifically addressed the question of whether the phase-delay part of the PRC shifts immediately after application of a phase-advancing light pulse. To answer this question, we determined the position of the PRC at the time of application of the second light pulse (Experiment 2) with the delay part of the PRC induced by a single light pulse (Experiment 3). The results are in accordance with previous studies (Best et al., 1999) and add to this that (a) the phase-delay part of the pacemaker shifts within one circadian cycle to its new steady-state position after a phase-advancing light pulse, (b) the magnitude of a transient shift predicts the phase of the new steady-state position, and (c) the waveform and amplitude of the phase-delay part of the PRC do not change after a phase-advancing light pulse.

PRC Amplitude and Waveform Stability

Amplitude

The question arises whether the properties of the pacemaker change during transient cycles. In fact, the data can only be interpreted on the premise that the pacemaker responds in its usual way to light during the transient cycles. Only then do the results from Experiment 3 form a reliable prediction for the pacemaker's responsiveness to the second light pulse.

Previous double-pulse experiments in invertebrates (*Drosophila*, Pittendrigh, 1979; *Neurospora*, Crosthwaite et al., 1995), and the Syrian hamster (Best et al., 1999) were performed to investigate if there is a refractory period in the pacemaker after application of a light pulse and to determine how long this refractory period lasts (i.e., how fast the pacemaker can react to a second light pulse). These data show that the pacemaker is capable of reacting to a new light pulse within a few hours. In other studies, however, it was shown that mice and hamsters are significantly less responsive to a second light pulse when applied within 4 h after the first light pulse (Khammanivong and Nelson, 2000; Nelson and Takahashi, 1999). Moreover, Khammanivong and Nelson (2000) indicated that responses are not even back to normal in the next circadian cycle (about 70% of expected shift). Our experiments show that the amplitude of the phase delay is back to normal 17 to 21 h after a phase-advancing light pulse. This raises the possibility that responsiveness to light is decreased 24 h after a light pulse but is unchanged 17 to 21 h after a light pulse. In other words, the part of the PRC that received the first light pulse is still affected by it after 24 h, whereas other parts of the PRC may be unaffected.

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Waveform

The waveform of the PRC appeared unaltered on the first cycle after the light pulse between CT 12 and CT 16. We have no knowledge of the phase response properties at other circadian times. Our data are consistent with unpublished data of Elliot and Pittendrigh (1996; Pittendrigh, 1981, Fig. 9). With respect to the phasedelay part of the PRC, they showed that between CT 12 and CT 16, the PRC had shifted to the steady-state position within one circadian cycle. However, they showed that the onset of the phase-delay part of the PRC (about CT 11) had not shifted. As a consequence, the phase-delay part of their PRC was compressed. We did not investigate responsiveness before CT 12 because we administered our second light pulse after activity onset of the animal to obtain an accurate prediction for the induced phase shifts.

Predicting Steady-State Phase Shift from the First Transient Cycle

The present analysis shows that there is a strong relationship between the position of the activity onset during the first and second transient cycles and the steady-state phase shift of activity onset (Fig. 3). This enabled us to make a very reliable estimate of the steady-state phase shift induced by the first light pulse in Experiment 2 on the basis of the first activity onset. The strong correlation indicates that the daily shifts in activity onsets during transient cycles are regulated with great precision and reflect the magnitude of the steady-state shift. Thus, the immediate shift is a fixed fraction of the steady-state shift. This indicates by itself that during the first transient cycle the steady-state phase shift is already determined. This is consistent with our conclusion that the pacemaker has reached its steady-state position on the first transient cycle.

This result is suggestive for an interaction between the endogenous pacemaker and a secondary downstream oscillatory system, either inside or outside the SCN, of which the kinetics can be described with great mathematical precision. We will discuss possible secondary systems in the next section.

Immediate Resetting

We showed that after a phase-advancing light pulse, the phase-delay part of the PRC shifts within one circadian cycle to the new steady-state position while the overt rhythm displays transients. If the pacemaker shifts immediately, the question arises, What process induces transient behavior? Transients in *Drosophila* have been attributed to the existence of two coupled oscillators of which one is light sensitive, whereas the other is temperature sensitive (Pittendrigh et al., 1958). In mammals, two mutually coupled circadian oscillators within the central pacemaker have been proposed (Pittendrigh and Daan, 1976). One oscillator is thought to lock onto the evening light (the E oscillator) and controls activity onset in nocturnal animals, while the other locks onto the morning light (the M oscillator) and controls activity offset. It has been suggested that the differential shift in onset and offset after a phase-advancing light pulse causes different shifts of E and M, with the M oscillator shifting immediately to its new steady-state position and E shifting more slowly (Honma et al., 1985; Meijer and Devries, 1995; Elliot and Tamarkin, 1994; Pittendrigh and Daan, 1976).

Accumulating evidence at a number of research levels supports the proposition, initially put forward by Pittendrigh and Daan (1976), that the circadian pacemaker is composed of different oscillators. Daan et al. (2001) summarized these results elegantly in a conceptual framework. The E and M components are thought to result from a double set of circadian genes. The M oscillator consists of *per1* and *cry1* and is accelerated by light, the E oscillator of *cry2* and *per2* and is decelerated by light. Evidence in favor comes, for instance, from Albrecht et al. (2001), who demonstrated that *per1* knockout mice lost the capacity to respond with advances to a light pulse, whereas *per2* knockouts no longer respond with phase delays (see Daan et al., 2001, for more details). An alternative model was proposed by Hastings (commentary to Daan et al., 2001). E and M could reflect *per1*/*per2* expression (M) versus *cry1*/*cry2* expression (E). This model is supported by differential peak times of the *per*s and *cry*s under different photoperiods.

The transients that are observed after a phase-advancing light pulse are attributed to an immediate shift of M (the oscillator that responds with advances to light) and a delayed shift of E, as a consequence of coupling forces. This explanation would also be consistent with Jagota et al. (2000), who demonstrated two peaks in multiunit activity in the SCN slice preparation. One peak occurred at the onset of dawn (possibly the M oscillator) and responded to glutamate with an immediate advance, while the other component did not respond. The evening rise and morning decline of melatonin show similar differences in their

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responsiveness to light. A phase-advancing light pulse results in an immediate advance of the melatonin decline and in a delayed advancing shift in melatonin rise (Elliot and Tamarkin, 1994; Illnerova, 1991). We have summarized the responses to phase-advancing light pulses in Table 1.

Best et al. (1999) demonstrated that the phase-advancing part of the PRC is reset within a few hours by light at CT 19. Our results demonstrate that within one circadian cycle light presentation at CT 19 also results in advances of the delay part of the PRC, at least of the delay part between CT 12 and CT 16. If the delay part of the PRC shows rapid resetting, similar to the advance part of the PRC, transients in the onset of activity cannot readily be explained on the basis of differential shifts of the E and M component when it is assumed that E is represented by the delay and M by the advance portion of the PRC.

Although our results do not follow directly from the proposed model of Daan et al. (2001), they are not necessarily in conflict with it either. An immediate shift of the M component (*per1*/*cry1*) in response to light at CT 19 may result in an immediate shift of the E component (*per2*/*cry2*) as a consequence of strong coupling forces between the two. The same reasoning is applicable to the model of Hastings (2001), but now with a different set of genes; an immediate shift of M (*per1*/*per2*) may result in a shift of E (*cry1*/*cry2*) within one cycle as a consequence of coupling. However, irrespective of the set of genes that may underlie E and M, our results suggest rapid resetting of both the delay and advance portions of the PRC.

A different viewpoint arises when data from intact animals are compared with data obtained in slice preparations. Phase resetting of the pacemaker has been studied in vitro by recording circadian rhythms in sampled single unit activity from SCN brain slice preparations. This activity displays a circadian rhythm, and the time of peak activity is used as a phase marker (Prosser and Gillette, 1989; Prosser, 1998). SCN brain slices can be kept in good condition and recorded from for two or three cycles. Phase shifts are commonly induced between the first and second cycle. Phase shift experiments in vitro have indicated that phase shifts in the SCN slice are immediate and stable (McArthur et al., 1991; Prosser, 1998; Watanabe et al., 2000) also when glutamate is administered at CT 19 (Ding et al., 1994). In these preparations, most downstream processes are eliminated because the slice is disconnected from most of its input and output. This indicates that transients could be the result of secondary processes, which are downstream from the pacemaker, outside the SCN.

Table 1. Phase shifts induced by a phase-advancing light pulse at CT 19. The table illustrates clear asymmetry in the effect of light on behavioral activity and melatonin (NAT) rhythms and on multiunit activity (MUA). Immediate shifts were obtained in activity offset, melatonin offset, and morning component of MUA. No immediate shifts were obtained in activity onset, melatonin onset, and in the evening component of MUA. No asymmetry exists for the shifts in advancing and delaying parts of the PRC, as both shift immediately. The shift of the rhythm in *mPer* is investigated in response to a 6-h advance of the light-dark cycle (and not in response to a short light pulse at CT 19) *mPer1* shifts immediately in response to this shifted cycle. It is unknown whether *mPer2* rhythms are immediately reset by phase-advancing stimuli.

	Response to Advancing Light Pulse at CT 19	
	Immediate Shift	*No Immediate Shift*
Behavioral activity rhythm offset		
Elliot and Tamarkin, 1994		
Meijer and De Vries, 1995	x	
Behavioral activity rhythm onset		
Elliot and Tamarkin, 1994		
De Vries and Meijer, 1995		x
Melatonin (NAT) offset		
Elliot and Tamarkin, 1994		
Illnerova, 1991	x	
Melatonin (NAT) onset		
Elliot and Tamarkin, 1994		
Illnerova, 1991		x
Multiunit activity peak dawn (M)		
Jagota et al., 2000	x	
Multiunit activity peak dusk (E)		
Jagota et al., 2000		x
Advance part PRC		
Best et al., 1999	x	
Delay part PRC		
This study, 2001	x	
mPer1 rhythm		
Yamazaki et al., 2000	x	
mPer2 rhythm	?	?

The latter offers an alternative explanation for the occurrence of transients. It has been proposed that there might be secondary oscillatory systems (slave oscillators) regulating the coupling of the activity rhythm with the circadian pacemaker (Pittendrigh and Daan, 1976; Takamure et al., 1991; Yamazaki et al., 2000). These secondary oscillators may require more than one circadian cycle to achieve complete re-entrainment. This hypothesis is supported by the finding that the velocity of re-entrainment of different circadian rhythms (urinary ion secretion, adrenocortical levels, core body temperature) after crossing several time zones is not the same (Klein et al., 1972; Moore-Ede et al., 1982; Van Cauter and Turek, 1986). Recently it was proposed that resetting of circadian

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time in peripheral tissue occurs via glucocorticoid signaling (Balsalobre et al., 2000), indicating that glucocorticoid is one of the possible candidates entraining secondary oscillators to the circadian pacemaker. Together the data indicate the existence of various time lags from the circadian pacemaker to each secondary oscillatory system.

The present experiments were not aimed to indicate where transients originate and leave open the possibility that behavioral transients are attributable to downstream processes, outside the SCN. The results have demonstrated that at least part of the pacemaker has shifted fully and within one circadian cycle in response to light. It is possible that the first part of the delaying area of the PRC (before CT 12) shows compression in response to a phase-advancing light pulse (see Pittendrigh, 1981) and that this accounts for transients in activity onset. This would lead to the conclusion that part of the delay curve shifted immediately, and part of it did not. The question of whether the pacemaker shows immediate resetting in response to light pulses should then be reformulated into a more specified question: Which parts of the pacemaker show immediate resetting and which parts do not? It may appear necessary to not only distinguish between the advance and delay areas of the PRC but even within these areas.

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