Light-Induced Phase Shifts in Onset and Offset of Running-Wheel Activity in the Syrian Hamster

Johanna H. Meijer and Martinus J. De Vries
Department of Physiology, University of Leiden, P.O. Box 9604, 2300 RC, Leiden, The Netherlands

Abstract The effects of light pulses on activity onset and offset were assessed in intergeniculate leaflet- and ventral lateral geniculate nucleus-lesioned Syrian hamsters with a precise onset and offset of circadian wheel-running activity. Light pulses applied to animals in constant darkness during the early subjective night induced phase delays in both activity onset and offset, while light pulses during the late subjective night induced phase advances in the onset and offset of activity. Despite the fact that the direction of onset and offset shifts were similar, differences were found in the magnitude of light-induced phase shifts. Steady state phase delays were larger in the activity onset, while steady state phase advances were largest in the offset of activity. We found phase delays and phase advances within one cycle after the presentation of a light pulse in both activity onset and offset. Differences in magnitude of these immediate phase shifts in activity onset and offset resulted in a compression of activity time for a number of cycles following a light pulse. Similar results were obtained in a selected group of intact animals indicating that intergeniculate leaflet- and ventral lateral geniculate nucleus-lesioned hamsters provide a good model to investigate the effects of light on circadian onset and offset of running-wheel activity.

Key words circadian rhythms, entrainment, light, phase response curve, suprachiasmatic nucleus

INTRODUCTION

A major circadian pacemaker in mammals is located in the suprachiasmatic nuclei (SCN) at the base of the anterior hypothalamus (Meijer and Rietveld, 1989). The SCN drive circadian rhythms in food and water intake, hormonal levels, sexual behavior, and locomotor activity (Meijer and Rietveld, 1989), and the pacemaker of the SCN is sensitive to light in a phase-dependent manner. Studies of the SCN pacemaker's phase shift response using wheel-running activity as the overt measure show that exposure to light during the early subjective night delays the pacemaker's rhythm, whereas at the end of the subjective night, light induces a phase advance (Daan and Pittendrigh, 1976; Takahashi et al., 1984). In consequence, the pacemaker entrains to the light-dark cycle. The effects of light on the phase of the pacemaker are summarized in a phase response curve (PRC).

Light information reaches the SCN via the retina. A direct pathway from the retina to the SCN, the retinohypothalamic tract (RHT), is sufficient for entrainment to occur (Johnson et al., 1988; Pickard et al., 1987). An indirect visual pathway to the SCN leads via the intergeniculate leaflet (IGL) and the ventral lateral geniculate nucleus (vLGN) of the thalamus (Morin et al., 1992), and is called the geniculohypothalamic tract (GHT). The GHT may contribute to photic entrainment (Harrington and Rusak, 1986; Johnson et al., 1989; Pickard et al., 1987; Rusak et al., 1989).

The two most important features used to investigate the circadian clock are its phase and period. These

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 10 No. 1, March 1995 4-16

can be measured irrespective of the organism or function that is studied. Model systems in this field are therefore powerful because they provide results that can be compared easily with results obtained in other species. The hamster is used as a model system in the study of mammalian circadian rhythms because it has an easily measurable rhythm in running-wheel activity. The regular and well-defined circadian onset of running-wheel activity provides a clear phase marker of the circadian pacemaker (Pittendrigh and Daan, 1976a) and is defined as circadian time (CT) 12. The offset of running-wheel activity, however, is often not so clear and shows a large variability from day to day (De Vries, 1992; De Vries and De Vries, in press; Pittendrigh and Daan, 1976a).

We have previously determined that lesions of the IGL and vLGN decrease the variability in running-wheel activity offset in hamsters (De Vries, 1992; De Vries and De Vries, in press). We thus obtained a second phase marker of the circadian pacemaker. We have now used these ablated animals to determine the effects of light pulses on the activity offset in detail and to compare these effects with light-induced phase shifts in the onset of activity. We paid special attention to the kinetics of onset and offset phase shifts.

MATERIAL AND METHODS

Animals

This study was performed on 11 male Syrian hamsters (*Mesocricetus auratus*, Harlan/CPB, Zeist, The Netherlands), which had previously received an electrolytic lesion aimed at the IGL and the vLGN of the thalamus. The animals were individually housed in cages ($l \times w \times h = 36.5 \times 25.0 \times 16.0$ cm) with a running wheel (diameter: 26.0 cm). The cages were kept in constant darkness (DD) in a sound-attenuating, ventilated room at a temperature of 23°C. At the beginning of the experiment, a computer system accumulated the amounts of activity over 30-minute periods. During the experiments, we switched to a system that recorded the presence or absence of running-wheel activity each minute. Food (Hope farms B.V.) and water were continuously available. The ablation procedure has been described previously (De Vries and De Vries, in press). In six of the animals, the IGL and vLGN were completely bilaterally ablated. In three animals the posterior IGLs were spared while in one animal the anterior vLGN and IGL were bilaterally spared. In one animal the nuclei were completely ablated on one side while minor damage was done to the contralateral IGL and vLGN. These lesions resulted in a very precise activity offset (De Vries, 1992; De Vries and De Vries, in press).

Experimental Light-Pulse Procedure

Animals were exposed to a 60-minute light pulse of saturating light intensity of 140-180 lx (Meijer et al., 1992) with an interval of at least 14 days. This was provided by moving the animal in its home cage to a place specially equipped to give light pulses. After a pulse the animal was returned to DD and was not disturbed for 14 days. Light pulses were applied during the animals' activity time (α) of the circadian cycle. Clean cages were provided only in between the experiments.

Data Analysis

Steady state and immediate phase shifts. The shape of the PRC varies with α (Daan and Pittendrigh, 1976; Goldman and Elliott, 1988; Honma et al., 1985; Stephan, 1983). Because we found a large variation in α among animals, we have expressed the timing of the light pulse as a fraction of α and not relative to activity onset (in CT). This seemed particularly appropriate as the effects of a light pulse on both activity onset and offset were investigated. We could now express the timing of a light pulse relative to both onset and offset. A functional reality for the %α time scale was furthermore derived from the clearly defined crossover from delay to advance shifts. This point was less clearly defined when a CT scale was used.

We have standardized α on the day before each light pulse (α_{before}) to 100%. α_{before} was defined as the distance between the fitted lines through activity onsets and offsets on the last day before the light pulse. The distance between the predicted activity onset and the midpoint of the light pulse was expressed as a percentage of α_{before}. The magnitude of the phase shifts (on the ordinate) were plotted as a function of the percentage of α_{before} (on the abscissa) in a phase response plot.

For pulses given during the first 40% of α, we measured the steady state and immediate phase shift in activity offset on the day of the pulse while the steady state and immediate phase shift in onset was measured on the next cycle (Fig. 1A). When light

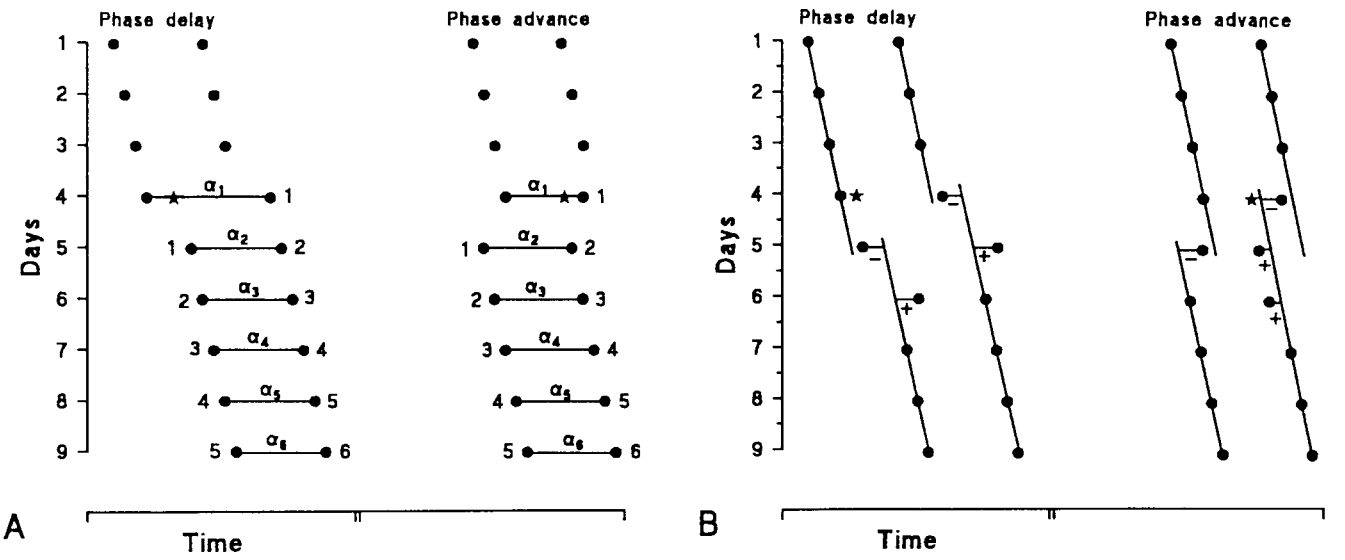

Figure 1. (A) Schematic drawing of onsets and offsets of running-wheel activity before and after a light pulse (indicated by an asterisk). The immediate phase delaying effect of a light pulse on the activity offset could be measured reliably on the same cycle, roughly 8-12 hours after the light pulse (offset No. 1). An effect of an advancing light pulse on the offset could also be measured on the same cycle (offset No. 1), approximately 0-7 hours after the pulse. However, this value is much affected by the light pulse presentation, especially when the pulse is given toward the end of α. For that reason, we defined phase shifts one cycle after the light pulse (offset No. 2) as the immediate phase advance of the activity offset. The immediate phase shifting effect of both delaying and advancing light pulses on the activity onset were measured on the next cycle (onsets No. 1). The immediate phase shifts were used to construct phase response plots. (B) Straight lines represent eye-fitted steady state regression lines through activity onsets and offsets before and after a light pulse (indicated by an asterisk). *Phase delays.* We measured the deviation of the observed activity onset (or offset) from the steady state regression line for 10 cycles. When the actual activity onset (or offset) on a given day after the light pulse is later than the activity onset (offset) as predicted from the regression line, the deviation is defined positive (and represents an "overshoot" in the phase shift). When the activity onset (offset) is earlier than predicted, the deviation is defined negative. *Phase advances.* When the activity onset (or offset) on a given day is later than the predicted activity onset (offset), the deviation is defined negative, and when the activity onset (offset) is earlier than predicted, it is defined positive.

pulses were given during the last 60% of α, steady state and immediate phase shifts in both activity onset and offset were measured on the next cycle (Fig. 1A). Straight lines were fitted by eye through the last seven activity onsets and offsets before a light pulse. Lines were also eye-fitted through steady state onsets and offsets up to the fourteenth cycle after the pulse. A number of transient cycles after the light pulse were excluded from the analysis of the steady state phase shift.

In order to determine the steady state phase shift, we extrapolated the fitted lines before and after the light pulse to the day of the measurement and determined the difference. We estimated the differences between the steady state phase shift in activity onset and offset for pulses given at those phases where phase delays ("early α" when 10-35% of α has passed) and where phase advances ("late α" when 50-90% of α has passed) were clearly obtained.

To determine immediate shifts, we measured the difference between the time of observed activity onset (or offset) and the time as predicted by the line through onsets (or offsets) before the pulse. For phase delays obtained during early α and advances obtained during late α, we investigated the differences between immediate and steady state phase shifts in both onsets and offsets. In particular, we investigated the differences between immediate phase shifts in activity onsets and offsets. For all comparisons we used a paired Student *t* test and set the level of significance at 0.05.

Deviations. When phase delays or advances were substantial, transients could be observed in onset and/or offset of activity. We have analyzed the transient behavior of the circadian pacemaker by measuring the difference between the actual activity onsets (or offsets) and the time of activity onset (offset), as predicted by the fitted line after the pulse (Fig. 1B). We

have done so for the first 10 onsets and offsets after the light pulse for delays (during early α) and advances (during late α). The deviation between the actual activity onset or offset and the predicted one was given a negative sign when the phase shift was not yet complete and a positive sign when the phase shift on that day was larger than predicted (Fig. 1B).

Activity time (α) and period (τ). α_{before} was defined above as the time between the prepulse onset and offset lines on the day before the pulse. We also measured α on the following 10 cycles as the time between observed activity onset and offset (Fig. 1A). For both phase delays (during early α) and phase advances (during late α), the first measurement of α was on the day of the pulse (α_1 in Fig. 1A). On each day, α was expressed as a percentage of α_{before}. The slopes of the fitted lines were used to calculate the period (τ) of activity onset (τ_{onset}) and offset (τ_{offset}). Estimates of differences between τ before and after a light pulse were assessed using a paired Student *t* test.

Compensation of α. We calculated the difference between the steady state phase shift of the onset and offset. We also calculated the difference between τ_{onset} and τ_{offset}. We have pooled the data from the phase delays and phase advances and plotted the difference in τ against the difference in phase shift. The relationship between them was analyzed using least squares linear regression.

Intact animals. Ten hamsters with activity offsets that were occasionally, for a period of several weeks or more, less variable, were selected out of a large group of intact animals. These hamsters received 15-min. phase advancing light pulses (of saturating intensity). In these animals we determined immediate and steady state phase shifts and light-induced changes in α according to the methods previously described.

RESULTS

Steady State Phase Shifts

A total of 64 light pulses were applied to 11 animals with clear onsets and offsets of activity. We found a large range of α (10.30-15.90 h, mean ± SEM: 12.96 ± 0.16 h) on the day before the pulse (α_{before}). We have standardized α_{before} to 100% and expressed the timing of the light pulse as a percentage of α_{before}. Light pulses given during the first 40% of α induced mostly phase delays in onset and offset of activity, whereas during the last 60% of α, they induced mostly advances. Inspection of the actograms reveals that shifts in onset and offset are in the same direction (Fig. 2). However, activity onset and offset can shift to a different extent (see Fig. 2). The magnitudes of steady state phase shifts have been plotted in a phase response plot (Fig. 3 A,C).

A quantitative analysis was performed on light pulses that induced clear phase delays (applied during early α, $n = 22$ pulses in 10 animals) or clear phase advances (applied during late α, $n = 24$ in 8 animals). Steady state phase delays were larger in onset (mean ± SEM: –1.90 ± 0.20 h) than in offset of activity (–1.43 ± 0.18 h, $p < 0.0001$), and steady state advances were larger in offset (3.33 ± 0.39 h) than in onset (2.31 ± 0.25 h, $p < 0.0005$) (Fig. 5).

Comparison of Immediate and Steady State Phase Shifts

Inspection of the actograms suggested that phase shifts in activity onset and offset can have different kinetics. Both activity onset and offset show an immediate phase delay when light pulses are presented during the first 40% of α. Immediate phase advances in onset and offset were induced by light pulses given during the last 60% of α (Fig. 3 B,D). In the remainder of this article, we analyzed the transient behavior of the onset and offset of activity in several ways.

Phase advances. Advances in activity onset are often accompanied by several transient cycles, but advances of the activity offset reach or exceed their steady state value within one cycle (Fig. 2). A comparison of immediate and steady state phase response plots suggests the same trend (Fig. 3). We have measured the difference between the real activity onset (or offset) and the steady state line for 10 cycles (Fig. 4).

On the first 3 days after the light pulse, we observed that the deviation of activity onsets was negative, indicating that the advance in activity onset was smaller than the steady state shift on those days. For activity offsets we found a negative deviation only on the day of the light pulse. However, this value should be interpreted with caution, because light pulse application toward the end of α interferes with the sponta-

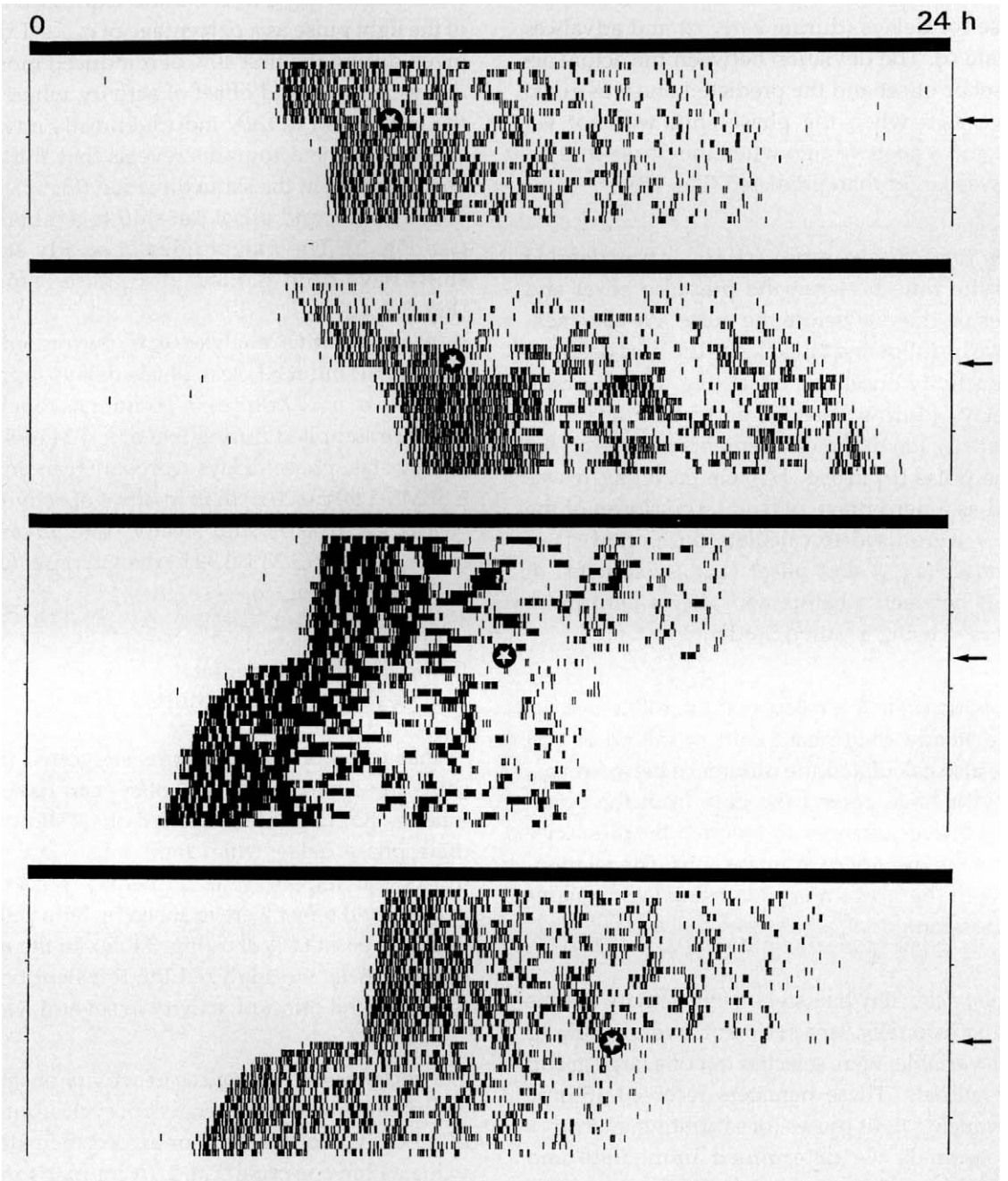

Figure 2. Running-wheel actograms of hamsters housed in DD. The time of day (in hours) is indicated above the actogram; the successive days are plotted beneath each other. A computer system recorded the presence or absence of running-wheel activity each minute. The days at which light pulses were applied are indicated by arrows; the time of a light pulse is indicated by an asterisk. Light pulses in the early subjective night induce phase delays of both the onset and offset of running-wheel activity. Light pulses during the late subjective night advance both the onset and offset of the activity rhythm.

neous offset of wheel-running activity. Therefore, we defined the immediate phase shift for offset advances as the shift on the cycle following the pulse. On this cycle, about 24 hours after the light pulse, the deviations of the offsets were negative (n = 4), zero (n = 5) or positive (n = 15). The means of these deviations remained positive for a number of days (Fig. 4), indicating that the means of the phase shifts during the first few days after the pulse were larger than that of the steady state phase shifts. The occurrence of an overshoot in the phase shift was not related to the timing of the pulse application. The differences be-

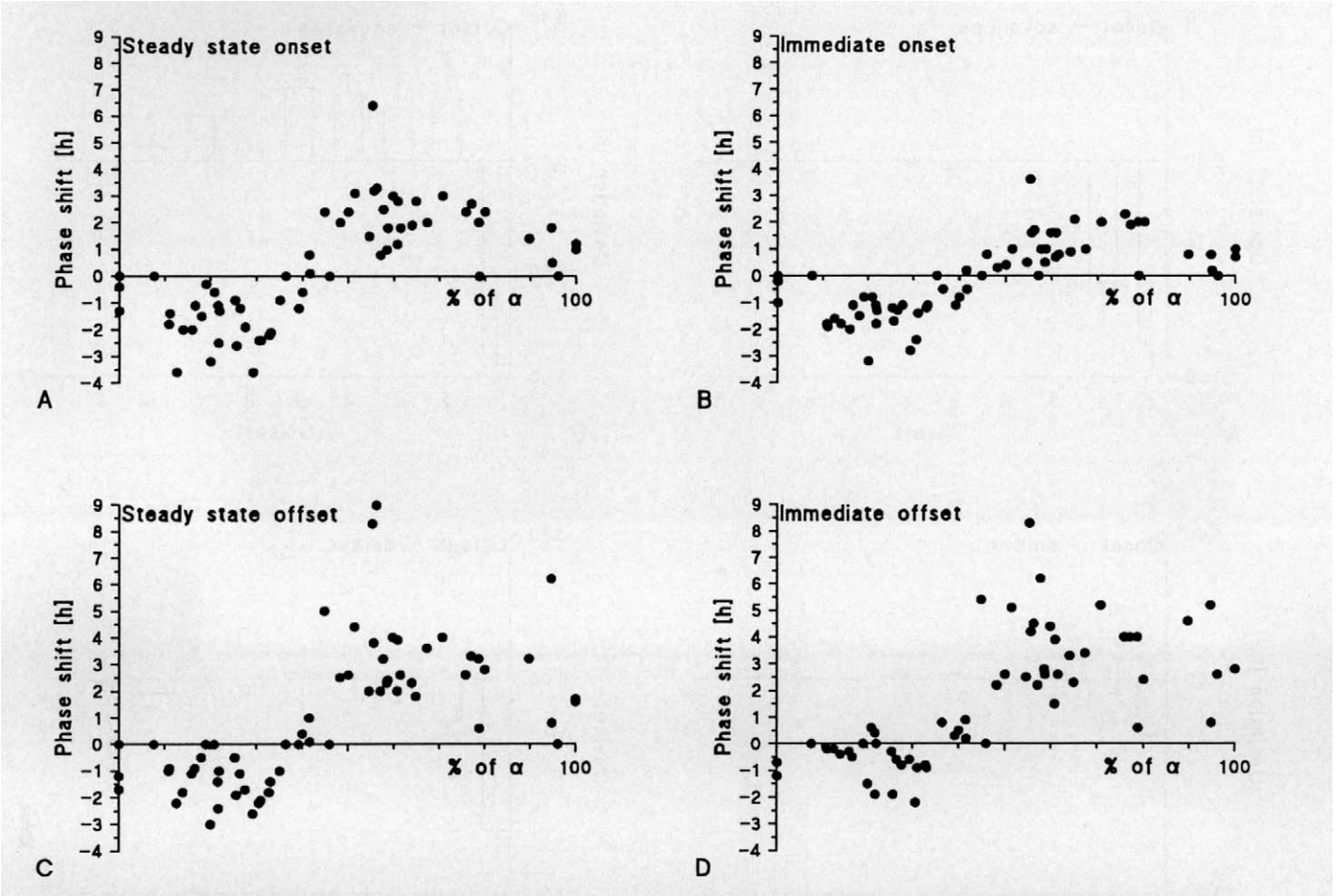

Figure 3. Phase response plots for the steady state (A) and immediate (B) phase shift of the activity onset, and for the steady state (C) and immediate (D) phase shift of the activity offset induced by a 60 min light pulse (140-180 lx). On the abscissa, the time of the light pulse presentation is given as a percentage of α_{before}. On the ordinate, phase advances are indicated in positive direction and delays in negative direction.

tween immediate and steady state phase advances were studied (Fig. 5). The immediate advance in the activity offset (3.63 ± 0.34 h) was not significantly different from the steady state value (3.33 ± 0.39 h), but the immediate phase advance in onset (1.29 ± 0.17 h) was smaller than the steady state phase advance (2.31 ± 0.25 h, $p < 0.0001$).

Phase delays. Immediate delays in onset and offset have been plotted in a phase response plot (Fig. 3 B,D). We found that the means of the deviations were negative in both activity onsets and offsets for a few cycles after the light pulse (Fig. 4). Analysis of individual examples indicates that delays in offset were mostly accompanied by negative deviations and that positive deviations were never observed (Fig. 2). In contrast, delays in onset were sometimes accompanied by positive deviations ($n = 5$), negative deviations ($n = 9$) or were completed within one cycle ($n = 8$). Despite this variability, the mean immediate phase delay was significantly smaller than the steady state delay for both activity onsets (–1.59 ± 0.13 vs. –1.90 ± 0.20 h, $p = 0.030$) and activity offsets (–0.65 ± 0.17 vs. –1.43 ± 0.18 h, $p < 0.0001$) (Fig. 5).

Comparison of Phase Shifts in Activity Onset and Offset

The immediate delay in activity offset appeared very small, as compared to the immediate delay in activity onset (Fig. 5). Immediate advances appeared larger in activity offset than in onset. The difference in the magnitude of immediate shifts in onset and offset was tested for those phases where large delays and

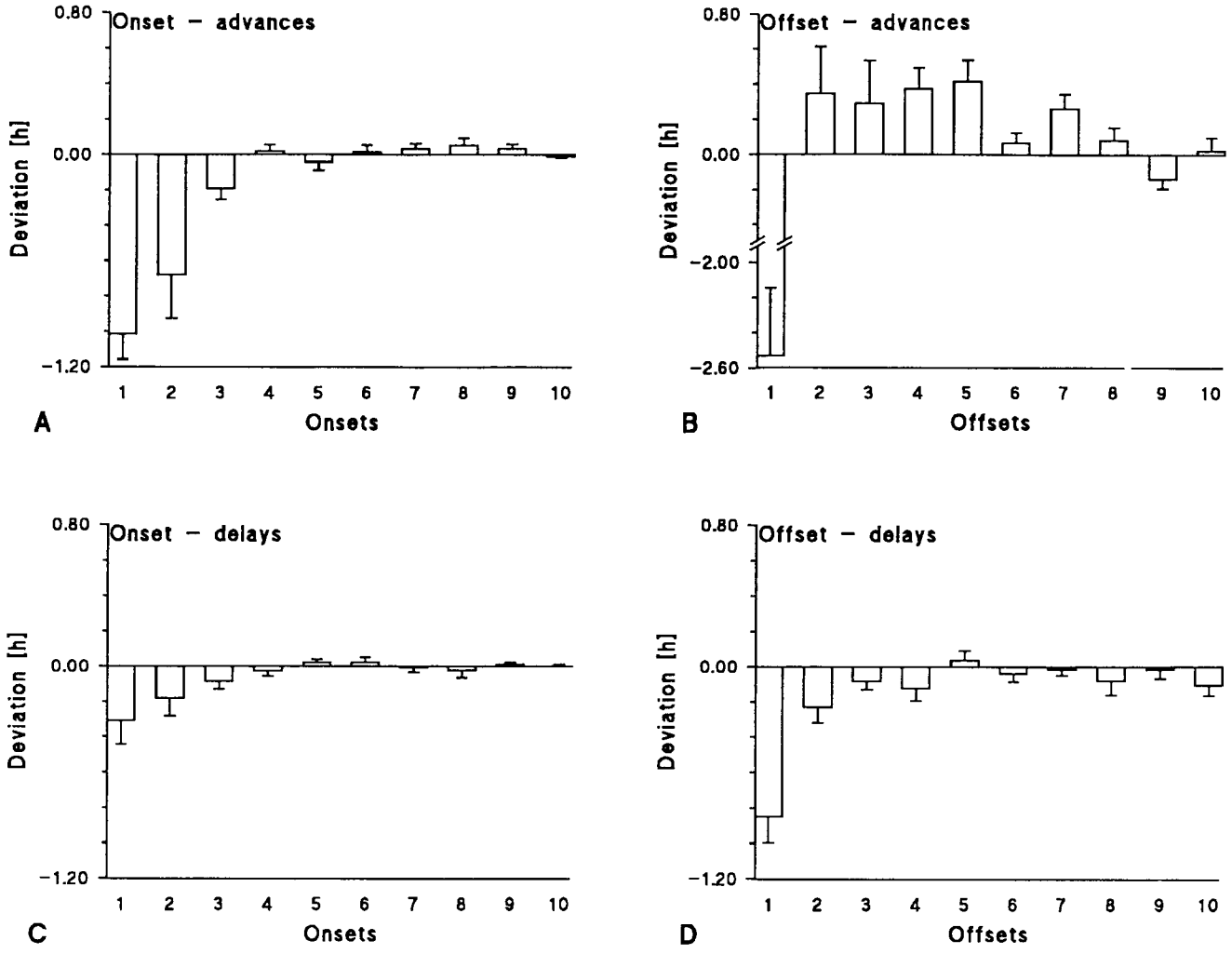

Figure 4. The deviations (mean ± SEM) of the actual activity onset and offset after the pulse from the fitted lines through the steady state values for the first 10 onsets and offsets following a light pulse. A: The deviation of the daily activity onset after a phase advance. B: The deviation of the daily offset after a phase advance. C: The deviation of the activity onset after a phase delay. D: The deviation of the offset after a phase delay.

large advances were obtained (Fig. 5). Immediate delays were larger in activity onset than in activity offset (–1.59 vs. –0.65 h, $p < 0.0001$), whereas immediate advances were larger in the offset than in the onset of activity (3.63 vs. 1.29 h, $p < 0.0001$).

Activity Time and Period of the Rhythms

A light pulse at the early subjective night induced an immediate phase delay in activity offset, resulting in a lengthening of α on that day (Fig. 6A). On the next cycle, α regained its original value (α_{before}), and on the following days, α became a little smaller. On the tenth cycle, α reached 97.3% ± 1.1% (SEM) of its original value (Fig. 6A). A light pulse at the late subjective night induced a phase advance (shortly after the light pulse) in offset of activity. As a result, α decreased on the day of the pulse (Fig. 6B). At the first cycle after the pulse, α appeared even smaller and reached its minimal value. At the following cycles, α approached to α_{before}, and at the tenth day, it was not significantly different from its original value (Fig. 6B).

After a phase delay, no significant effect on the steady state τ_{onset} was observed ($\tau = 24.07$ vs. 24.06). In contrast, τ_{offset} decreased significantly ($\tau = 24.09$ vs. 24.06, $p = 0.028$). After a phase advance, a significant decrease in τ_{onset} was found ($\tau = 24.10$ vs. 24.07, $p < 0.004$), while τ_{offset} changed not significantly ($\tau = 24.11$ vs. 24.14).

It is possible that differences in τ_{onset} and τ_{offset} have occurred to compensate for changes in α when steady

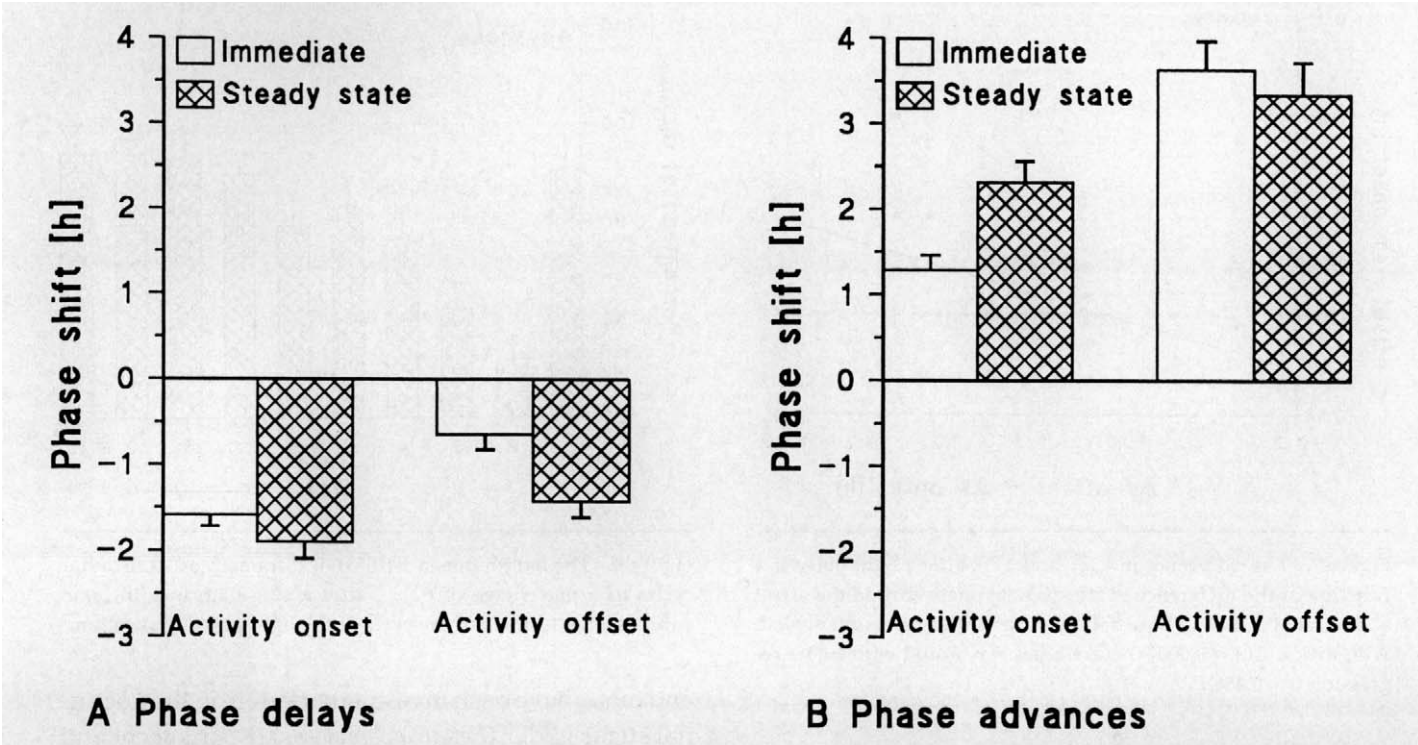

Figure 5. Mean (± SEM) immediate and steady state phase delays (A) (n = 22) and phase advances (B) (n = 24) in the activity onset and offset. All phase shifts, in both onset and offset, were significantly different from zero ($p \leq 0.001$). For mutual comparisons, see text.

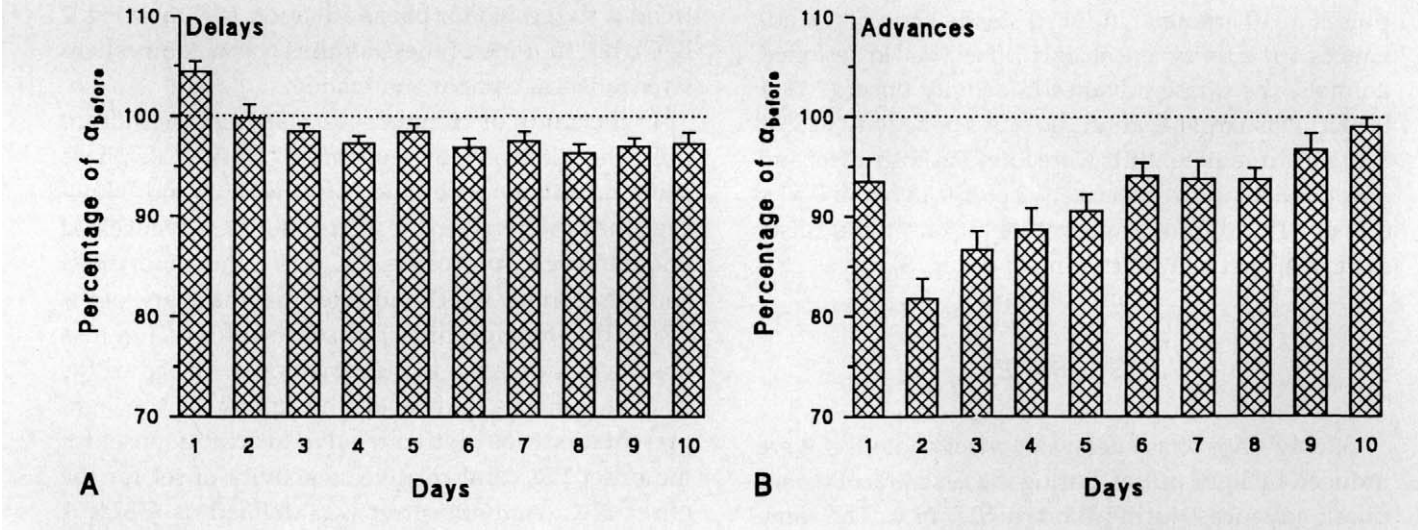

Figure 6. The length (mean ± SEM) of α for each of 10 circadian cycles as a percentage of α_{before} after phase delays (A) and phase advances (B). Day 1 indicates the cycle of the light pulse application.

state shifts in activity onset and offset were of different magnitude. The difference in τ_{onset} and τ_{offset} was therefore plotted as a function of the difference in steady state phase shifts in onset and offset (Fig. 7). The values from phase delaying and advancing light pulses were pooled. By linear regression, a significant relation was observed (r = 0.486, $p < 0.001$). In this analysis, one extreme value (5.70 h, 0.45 h) has been excluded. When

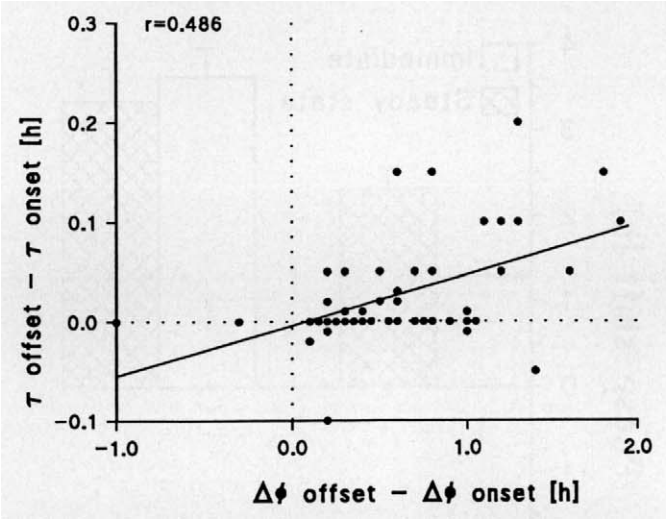

Figure 7. The difference in τ_{offset} and τ_{onset} after a light pulse as a function of the difference in steady state phase shift in the offset and onset of activity. Phase delays and phase advances are pooled. A significant (p = 0.00071) correlation was found with linear regression (r = 0.486).

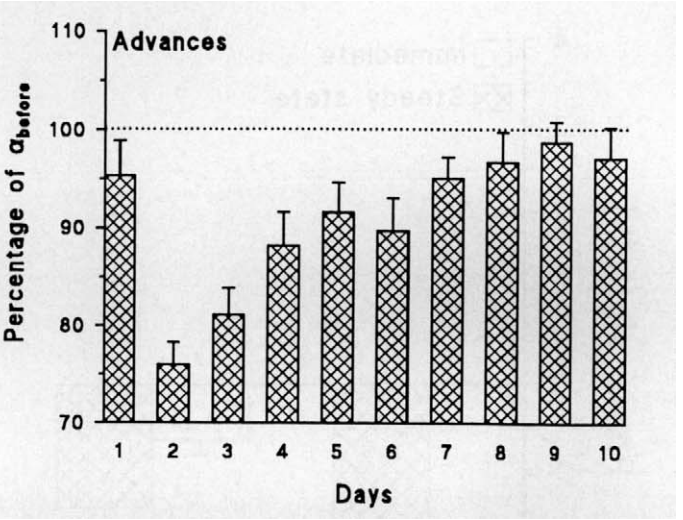

Figure 8. The length (mean ± SEM) of α for each of 10 circadian cycles as a percentage of α_{before} after a phase advance in intact animals. Day 1 indicates the cycle of the light pulse application.

this value was included in the linear regression, we found a correlation coefficient of 0.792 ($p < 0.00001$).

Intact Animals

In intact animals, light pulses during late α (10 pulses in 10 animals) induced steady state phase advances in activity onset and offset. As in lesioned animals, the phase advance in activity offset (2.19 ± 0.20 h) was larger than in the onset of activity (0.85 ± 0.10 h). Immediate shifts were found in both offset and onset of activity (respectively, 2.65 ± 0.18 h and 0.53 ± 0.13 h). The different magnitude of onset and offset shifts resulted in a shortening of α (Fig. 8).

DISCUSSION

Steady state phase delays in onset and offset were induced by light pulses during the first 40% of α, and phase advances during the last 60% of α. The same transition point was found in the phase response plot for steady state shifts in activity onset and offset, and no qualitative difference was observed in the shape of the phase response plot. However, quantitative differences were observed. Phase delays were largest in the onset, and phase advances were the largest in the offset of activity. The effects of light on activity onset and offset have been investigated before in the nocturnal sugar glider (*Petaurus breviceps*) (Kleinknecht and Erkert, 1991), in the hamster (Elliott and Tamarkin, 1994), and in the rat (Honma et al., 1985). Consistent with our results, the first study describes that onset and offset can shift by different amounts with larger delays in the onset and larger advances in the offset. For the hamster (Elliott and Tamarkin, 1994), the same trend is suggested for phase advances (see their Fig. 2 B, C), but in none of these studies was a comprehensive statistical comparison made.

In the study of Honma et al. (1985), no significant differences between the maximum steady state phase shifts in activity onset and offset were found. However, the ratio between the area under the advance and under the delay part of the PRC was different for onset and offset shifts, which indicates that the shape of the PRC is different for shifts in onset and offset. They also observed a difference in the transition point of the PRC of 1 or 2 hours. However, the timing of the light presentation was plotted relative to activity onset for the onset PRC and relative to activity offset for the offset PRC. Activity offset was defined as CT24. A quantitative comparison of transition points between the onset and offset PRC is valid only when α is exactly 12 circadian hours. Their mean α was 13.15 circadian hours. Thus a light pulse presented at, for instance, CT18 in their onset PRC falls 1.15 circadian hours earlier than a light pulse presented at CT18 in the offset PRC. When the timing of the light pulse is expressed

in terms of α, the difference in transition points of the two PRCs of Honma et al. (1985) becomes very small.

Immediate phase shifts in activity onset were measured one cycle after the pulse. It is clear that the onset can be advanced and delayed within one cycle. This is consistent with data in the hamster but contrasts with results in the rat, where immediate phase delays but no phase advances occurred in the onset of activity (Elliott and Tamarkin, 1994; Honma et al., 1985). For the first 10 days after the pulse, we determined the actual activity onset and measured the deviation from the steady state line. Negative deviations were found on a number of cycles for both advances and delays. For onset advances, the mean values were representative for the whole group. For delays, this was not the case, and we found a number of clear positive deviations. The occurrence of an overshoot in the phase delay was not related to the timing of the pulse and did not depend on the animal. Although our immediate shifts in activity onset differed significantly from zero, they also differed from the steady state phase shifts for both advances and delays. Thus immediate shifts in the onset reflect transient cycles.

Immediate delays in the offset of activity occurred on the day of the pulse (roughly 12 hours after the pulse) in the present study but not in a previous one (Elliott and Tamarkin, 1994). Our shifts were different both from the steady state phase shift and from zero, indicating the existence of a transient cycle. Deviations from steady state phase shifts remained negative for some days after the pulse. Immediate advances in activity offset are already completed within one cycle, consistent with Elliott and Tamarkin (1994). In fact, our immediate advances of the offset were often bigger than the steady state ones. Positive deviations were observed for a number of days (Fig. 4). It is important to note here that an earlier time of activity offset was already observed on the day of the pulse (mean shift ± SEM: 0.8 ± 0.25 h, $p < 0.005$). Deliberately, we have not defined this as an (immediate) phase shift because the value may be largely changed in either direction due to the prior manipulation of the animal. We have inspected individual actograms and found that advancing light pulses that were given rather early (when 50-70% of α has passed), and that may not have interfered with spontaneous activity offset, induced clear advances of activity offset. Our interpretation of the advanced offsets, on the day of the pulse, is that they represent true phase shifts that occurred within a few hours after the light pulse. However, the measured magnitude of this phase shift may be an underestimate of its real value because a large phase advance will bring the activity offset to a point where the animal had already been active. In that case, a large phase shift is not measurable.

The magnitudes of immediate phase delays in onset were compared with the offset delays. Immediate delays in the onset of activity were larger than in the offset. It could be questioned whether this measurement was biased because onset delays were measured about one cycle after the pulse, whereas offset delays were measured approximately half a cycle after the pulse and therefore may have had less time to shift. However, the immediate onset delay (Fig. 1A: onset No. 1) also differed ($p = 0.011$) from the next offset delay, one and a half cycle after the pulse (Fig. 1A: offset No. 2).

Immediate advances in activity offset were much larger than the immediate advances in onset of activity. As with delay shifts, the measurements of onset and offset advances were at different times after the pulse. The activity offset (measured one cycle after the pulse) may have had more time to shift than the onset (measured half a cycle after the pulse). However, the difference between the immediate offset advance and the next onset advance (Fig. 1A: onset No. 2) is still significant ($p < 0.0001$). Hence it is proved that immediate delays in the onset are larger than in offset, whereas, vice versa, immediate offset advances are larger than immediate onset advances.

The difference between the immediate shifts in onset and offset of activity results in a temporary change in α. For delays, a lengthening of α is present at the day of the pulse. This is easily understood because on this day only the offset could have been delayed. During the following days, α has shortened, which is caused by the fact that the transient delays in the offset were often smaller than in the onset. The time interval between onset and offset of activity thereby slightly decreases. Consistent with Elliott and Tamarkin (1994), we obtained a substantial shortening of α after advancing light pulses. Light pulses during the latter part of α induced an immediate advance of activity offset so that α decreased on the first day. On the second day, α decreased even more due to an even larger advance of activity offset and a smaller advance of the activity onset.

Besides the large temporary changes in α, which resulted from the large differences between onset and offset shifts for the first cycles after the pulse, some-

what smaller changes in α remained. We have plotted the difference in steady state phase shifts against the difference in τ_{onset} and τ_{offset} and found a significant positive correlation. Our interpretation is that the difference in τ_{onset} and τ_{offset} compensates for the change in α and that, as a consequence of different τs in onset and offset, the initial magnitude of α is finally restored.

Our hamsters had previously received geniculate lesions, which allowed us to follow shifts in activity onset and offset with much greater precision than in intact animals. The question is justified whether results from IGL- and vLGN-ablated animals can be generalized to intact animals. Indeed, we obtained qualitatively the same results in intact animals. Both steady state and immediate phase advances were larger in the offset than in the onset of activity. As reported previously for intact hamsters (Elliott and Tamarkin, 1994) and confirmed in our ablated animals (Fig. 6), a clear compression of α was visible for a number of days after a light pulse. We conclude that IGL- and vLGN-lesioned animals provide a good model to compare the effects of light on activity onsets and offsets.

The results have clearly indicated that onset and offset can shift to a different extent and with a different rate. The difference in onset and offset shifts might indicate that different oscillators control onset and offset of activity. Very likely, the SCN pacemaker consists of a large number of oscillators. Partial lesions of the SCN (Davis and Gorski, 1984; Pickard and Turek, 1985; Rietveld, 1984) as well as recordings from SCN slices or cultures (Green and Gillette, 1982; Groos and Hendriks, 1982; Watanabe et al., 1993) have all indicated that small parts of the SCN are capable of producing a circadian rhythm. A specific hypothesis that the SCN functionally consists of two (groups of) oscillators was formulated by Pittendrigh and Daan (1976b). According to their model, one oscillator locks onto the evening light (the E component) and controls the activity onset while the other locks onto the morning light (the M component) and controls the activity offset (Pittendrigh and Daan, 1976b). The two oscillators are thought to be mutually coupled.

This model is supported by its ability to explain the change in α when the length of day changes. Moreover, the model can account for splitting, since it has been suggested that the two components of the split activity rhythm are generated by the E and M oscillators (Pittendrigh and Daan, 1976b). However, the interpretation that E and M components of the split rhythm functionally correspond to the same E and M oscillators hypothesized to regulate, respectively, the onset and offset of activity in DD is largely phenomenological. It is derived by tracing back the split components of the hamster's activity rhythm to the onset and offset of activity (Earnest and Turek, 1982; Pittendrigh and Daan, 1976b). In other studies it could not be determined which of the split components stems from the onset and which from the offset of activity (Boulos and Morin, 1985; Lees et al., 1983; Morin and Cummings, 1982).

If it is true that components E and M correspond to the onset and offset of activity, respectively, a single light pulse may hit the two oscillators at a slightly different phase. It is then expected that even a small phase angle difference between the oscillators will result in a different effect of a light pulse on their phases. One piece of evidence for this stems from double pulse experiments of Elliott and Pittendrigh (Pittendrigh, 1981) in the golden hamster, in which it was shown that the advance part of the PRC was shifted more than the delay part following a light pulse at CT19. Pittendrigh (1981) concluded "it is as though only the morning (M) oscillator was immediately advanced and the evening (E) oscillator will require several other cycles before it regains a steady-state phase relation (ψEM) with its coupled partner" (p. 121). Other evidence stems from studies on the effects of light on the pineal enzyme N-acetyltransferase (NAT) in the rat (Illnerova, 1991) and melatonin and activity in the hamster (Elliott and Tamarkin, 1994), where it was found that the phase of the evening rise and morning decline of NAT and melatonin can be differentially affected by a single light pulse and may be controlled by two different oscillators.

Our results are also explicable in terms of such a model. The differential shifts of onset and offset would indicate that a single light pulse may indeed have hit the E and M oscillator in such a way that they shift differently, either because they are slightly out of phase and/or because their PRCs for light are different. PRCs for immediate shifts are often interpreted as if they reflect the direct effects of light on the oscillators. Our PRCs for immediate shifts may be taken to indicate that E and M can both phase delay and phase advance, since immediate phase delays and advances were observed in both the activity onset and offset. This is different from results on NAT and activity measurements in the rat (Honma et al., 1985; Illnerova, 1991) where no immediate advances were observed in the activity onset and NAT rise. Although we did observe immediate onset advances, the magnitude

was much smaller than the steady state onset advance and the immediate offset advance. This is consistent with the results in the hamster described by Elliott and Tamarkin (1994) who also described small but immediate advances in the onset of activity.

In our study, the activity offset was already delayed the same cycle at which a phase delaying pulse was presented, whereas in the study of Elliott and Tamarkin (1994), no phase delay was observed in the offset on the day of the pulse. We do not know how to explain the difference, but it may be related to the timing of the light pulses. One possibility is that Elliott and Tamarkin presented phase delaying pulses only at CT13. In our phase response plot for immediate phase delays in the offset (Fig. 3D), it seems that immediate delays are induced mostly by light pulses that are given during the later part of the delaying section of the phase response plot. Our results suggest that the E and M oscillator can both be phase delayed and phase advanced and thus have both a PRC with a delay and an advance part. Our results may be in agreement with results on hamsters or Tupajas with split rhythms. Immediate phase shifts induced by dark pulses (Boulos and Rusak, 1982; Lees et al., 1983), carbachol (Meijer et al., 1988), cycloheximide (Wollnik et al., 1989), or light pulses (Meijer et al., 1990) all produce similar PRCs for the two components of the split rhythm.

We would, however, like to be cautious in interpretation of the PRCs, because both the measured steady state and immediate phase shifts may have been subject not only to the effects of light but also to coupling forces between the oscillators (Pittendrigh and Daan, 1976b). For instance, when a phase advance occurs and the offset shifts immediately, it is unknown whether the small phase advance that we measure in the activity onset reflects the effect of light on the E oscillator or whether the shift is already a consequence of coupling forces with the M oscillator.

Finally, we would like to mention that our discussion of the two-oscillator model has been based on the assumption that running-wheel activity reflects the pacemaker's motion itself. Although this is well accepted for steady state phase shifts in running-wheel activity and for τ, it may not be so for transient cycles (Pittendrigh et al., 1991). The difference in kinetics between onset and offset shifts is not necessarily a pacemaker property. Since there is currently no reason to attribute more value to the activity onset than to the offset as a phase marker of the circadian pacemaker, it is concluded that the conventional analysis of onset shifts provides an incomplete picture of the pacemaker's response to a perturbing stimulus.

ACKNOWLEDGMENTS

We would like to thank Hans-Jaap Oppenheim for his assistance with the data analysis and Menno Gerkema for his active feedback. We thank Mervyn Wise for his comments on the manuscript. This work was supported by PSYCHON (Grant 560-258-046), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

REFERENCES

Boulos Z and Morin LP (1985) Entrainment of split circadian activity rhythms in hamsters. J Biol Rhythms *1*: 1-15.

Boulos Z and Rusak B (1982) Phase-response curves and the dual-oscillator model of circadian pacemakers. In *Vertebrate Circadian Rhythms: Structure and Physiology*, J Aschoff, S Daan, and G Groos, eds, pp 215-223, Springer, Berlin Heidelberg, New York.

Daan S and Pittendrigh CS (1976) A functional analysis of circadian pacemaker in nocturnal rodents. II. The variability of phase response curves. J Comp Physiol *108*: 253-266.

Davis FC and Gorski RA (1984) Unilateral lesions of the hamster suprachiasmatic nuclei: Evidence for redundant control of circadian rhythms. J Comp Physiol *154*: 221-232.

De Vries MJ (1992) The relation between the extent of intergeniculate leaflet and ventral lateral geniculate nucleus lesions and the variability of the activity offset in the Syrian hamster. J Interdiscipl Cycle Res *23*: 155-156.

De Vries MJ and De Vries AJ (in press) A change in the pattern in circadian running-wheel activity after lesions of the intergeniculate leaflet and ventral lateral geniculate nucleus in the Syrian hamster. Biol Rhythm Res.

Earnest DJ and Turek FW (1982) Splitting of the circadian rhythm of activity in hamsters: Effects of exposure to constant darkness and subsequent re-exposure to constant light. J Comp Physiol A *145*: 405-411.

Elliott JA and Tamarkin L (1994) Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. J Comp Physiol A *174*: 469-484.

Goldman BD and Elliott JA (1988) Photoperiodism and seaonality in hamsters: Role of the pineal gland. In *Processing of Environmental Information in Vertebrates*, MH Stetson, ed, pp 203-218, Springer Verlag, New York.

Green DJ and Gillette M (1982) Circadian rhythm of firing rate recorded from single cell in the rat suprachiasmatic brain slice. Brain Res *245*: 198-200.

Groos GA and Hendriks J (1982) Circadian rhythms in electrical discharge of rat suprachiasmatic neurons recorded in vitro. Neurosci Lett *34*: 283-288.

Harrington ME and Rusak B (1986) Lesions of the thalamic intergeniculate leaflet alter hamster circadian rhythms. J Biol Rhythms *1*: 309-325.

Honma K-I, Honma S, and Hiroshige T (1985) Response curve, free-running period, and activity time in circadian locomotor rhythm of rats. Jpn J Physiol *35*: 643-658.

Illnerova H (1991) The suprachiasmatic nucleus and rhythmic pineal melatonin production. In *The Minds Clock*, DC Klein, RY Moore, and SM Reppert, eds, pp 197-216, Oxford University Press, New York.

Johnson RF, Moore RY, and Morin LP (1988) Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Res *460*: 297-313.

Johnson RF, Moore RY, and Morin LP (1989) Lateral geniculate lesions alter hamster circadian activity rhythms in the hamster. Brain Res Bull *22*: 411-422.

Kleinknecht S and Erkert HG (1991) Asymmetric re-entrainment and phase response of the circadian activity rhythm of a nocturnal marsupial (Petaurus breviceps: Phalangeridae). J Interdiscipli Cycle Res *22*: 341-353.

Lees JG, Hallonquist JD, and Mrosovsky N (1983) Differential effects of dark pulses on the two components of split circadian activity rhythms in golden hamsters. J Comp Physiol A *153*: 123-132.

Meijer JH, Daan S, Overkamp GJF, and Hermann PM (1990) The two-oscillator circadian system of tree shrews (Tupaia belangeri) and its response to light and dark pulses. J Biol Rhythms *5*: 1-16.

Meijer JH and Rietveld WJ (1989) Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. Physiol Rev *69*: 671-707.

Meijer JH, Rusak B, and Gänshirt G (1992) The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms. Brain Res *598*: 257-263.

Meijer JH, Van Der Zee EA, and Dietz M (1988) The effects of intraventricular carbachol injection on the free-running activity rhythm of the hamster. J Biol Rhythms *3*: 333-348.

Morin LP, Blanchard J, and Moore RY (1992) Intergeniculate leaflet and suprachiasmatic nucleus organization and connections in the golden hamster. Vis Neurosci *8*: 219-230.

Morin LP and Cummings LA (1982) Splitting of wheel-running activity rhythms by castrated or steroid treated male and female hamsters. Physiol Behav *29*: 665-675.

Pickard GE, Ralph MR, and Menaker M (1987) The intergeniculate leaflet partially mediates effects of light on circadian rhythms. J Biol Rhythms *2*: 35-56.

Pickard GE and Turek FW (1985) Effects of partial destruction of the suprachiasmatic nuclei on two circadian parameters: Wheel-running activity and short-day induced testicular regression. J Comp Physiol *156*: 803-815.

Pittendrigh CS (1981) Circadian systems: Entrainment. In *Handbook of Behavioral Neurobiology, Biological Rhythms*, Vol 4, J Aschoff, ed, pp 95-123, Plenum Press, New York.

Pittendrigh CS and Daan S (1976a) A functional analysis of circadian pacemakers in nocturnal rodents. I. The stability and lability of spontaneous frequency. J Comp Physiol A *106*: 223-252.

Pittendrigh CS and Daan S (1976b) A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. J Comp Physiol *106*: 333-355.

Pittendrigh CS, Kyner WT, and Takamura T (1991) The amplitude of circadian oscillations: Temperature dependence, latitudinal clines, and the photoperiodic time measurement. J Biol Rhythms *6*: 299-313.

Rietveld WJ (1984) The effects of partial lesions of the hypothalamic suprachiasmatic nucleus in the circadian control of behavior. Ann Rev Chronopharmacol *1*: 1-3.

Rusak B, Meijer JH, and Harrington ME (1989) Hamster circadian rhythms are phase-shifted by electrical stimulation of the geniculo-hypothalamic tract. Brain Res *493*: 283-291.

Stephan FK (1983) Circadian rhythms in the rat: Constant darkness, entrainment to T cycles and to skeleton photoperiods. Physiol Behav *30*: 451-462.

Takahashi JS, Decoursy PJ, Bauman, and Menaker M (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature *308*: 186-188.

Watanabe K, Koibuchi N, Ohtake H, and Yamaoka S (1993) Circadian rhythms of vasopressin release in primary cultures of rat suprachiasmatic nucleus. Brain Res *624*: 115-120.

Wollnik F, Turek FW, Majewski P, and Takahashi JS (1989) Phase shifting the circadian clock with cycloheximide: Response of hamsters with an intact or a split rhythm of locomotor activity. Brain Res *496*: 82-88.