PHASE-SHIFTING EFFECTS OF DUSKLIKE AND DAWNLIKE LIGHT PULSES ON THE CIRCADIAN ACTIVITY RHYTHMS OF SYRIAN HAMSTERS

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ABSTRACT

This study tested whether light pulses with a dusklike offset or a dawnlike onset caused phase shifts of different sizes in the circadian wheel-running activity of Syrian hamsters, *Mesocricetus auratus*. Six experiments were conducted, each with 30 hamsters; the hamsters received first one type of pulse and then the other type a few weeks later, allowing a paired comparison. The six experiments represented the combination of two maximum light intensities (150 and 250 lux) and three zeitgeber times (ZTs) at which the pulses were given (ZT13.5, ZT14.5, and ZT20). Pulses were 30 minutes long, a relatively short duration to minimize circadian time effects. Aschoff's type II method of measuring phase shifts was used. In none of the six experiments did a two-tailed paired t test detect a significant difference in the size of phase shifts caused by dusklike versus dawnlike pulses. A three-way analysis of variance (ANOVA) on the combined data from all six experiments (with pulse type, pulse intensity, and ZT as factors) also failed to detect a significant effect of pulse type. Statistical power was calculated and found to be reasonably good. These negative results are in line with those of a previous study in which a different methodology was used. (*Chronobiology International, 18(3), 413–421, 2001*)

Key Words: Circadian activity rhythm; Dawn; Dusk; Hamster; Phase shift; Twilight

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INTRODUCTION

One of the fundamental properties of the circadian clock is its ability to phase shift in response to pulses of light delivered during subjective night. Various parameters can characterize these pulses and affect the magnitude of the ensuing shifts. It is well known, for example, that larger phase shifts are obtained after exposure to pulses that are longer or brighter (1) or of certain wavelengths (2,3). One parameter that has received little attention until recently, however, is the profile of light onset and offset during the pulse. Almost all studies so far have used "rectangular" pulses, with abrupt lights-on and lights-off events. There is little information on whether light pulses that simulate dawn or dusk have different effects on the magnitude of phase shifts. This is despite the fact that, in some species—but not all (4)—simulated twilights improve the strength of light-dark (LD) cycles as zeitgebers (5).

The Syrian hamster, *Mesocricetus auratus*, is one species for which the presence of twilight transitions promotes circadian entrainment, at least to long light-dark cycles (5). The hamster is also the only species, as far as we know, for which complete phase-response curves (PRCs) to dawnlike and dusklike light pulses have been established (6). A study by Boulos et al. (6) found little difference between dawnlike and dusklike pulses in the size of the phase shifts they caused. This is somewhat surprising. One possible mechanism by which twilight could promote entrainment to long light-dark cycles is one by which dusk transitions would cause larger phase delays, and if this is true, then one would expect dusklike pulses to have larger phase-delaying effects than dawnlike pulses.

In the present study, we used a protocol different from that of Boulos et al. (6) to test anew the hypothesis that dusklike and dawnlike pulses should have different phase-shifting effects. As in the study of Boulos et al., we worked with hamsters, but we introduced the following variations in our experimental protocol: We preexposed the hamsters to dawn and dusk during the entrainment phase of the study, before any phase shift was measured; we used a different method of obtaining and measuring phase shifts that allowed us to compare pulses at precisely the same zeitgeber time in the same animal. Also, we used light profiles that were of different duration (30 minutes instead of 60 minutes) and maximum light intensity (150 or 250 lux instead of 10 lux). Our main working hypothesis was that dusklike pulses delivered in the first quarter of the subjective night should lead to larger phase delays than dawnlike pulses given the better entrainment observed to long light-dark cycles that include simulated twilights and given also that dusk represents a more natural event than dawn in early night. We also explored the mirror-image possibility that dawnlike pulses delivered in the second half of the night could lead to larger phase advances than dusklike pulses.

EXPERIMENTAL

Animals and Setup

Hamsters (male, approximately 60 days old at the start of each experiment) were obtained from Charles River Canada (St. Constant, Quebec). On arrival in the laboratory, they were placed individually in polystyrene cages (48 × 27 × 20 cm) supplied with wood chips as litter. Water and food pellets were available ad libitum in those cages throughout each experiment. Each cage was also equipped with a running wheel 34.3 cm in diameter.

For 14–21 days, hamsters were left under a constant temperature of 22°C and a photoperiod of 14 hours of light and 10 hours darkness (14:10). Lights did not go on and off abruptly. Instead, a twilight simulator (SunMatch, Aquabiotech, Coaticook, Quebec) gradually increased and decreased the voltage delivered to 8 incandescent 100-W lights in the experimental rooms to simulate dawn and dusk. We programmed the simulator so that the gradual increase and decrease lasted 30 minutes. Dawn started from full darkness at 1:45h and reached full illumination at 2:15h, while dusk started from full illumination at 15:45h and reached full darkness at 16:15h. This can be said to correspond to LD 14:10 if we consider the temporal middle of dawn and dusk as the start and end points, respectively, of the light period.

The reason for using a twilight duration of 30 minutes was to mimic the duration of the future light pulses. The reason for having the light pulses last 30 minutes, rather than 60 minutes as in the study of Boulos et al. (6), was to decrease the difference in the amount of light impinging on the circadian clock at different circadian times (CTs). It is well known from the PRCs to light pulses that light falling at different CTs causes phase shifts of different magnitude (10). Our light pulses started at the same time within the same experiment, but because light in the dawn pulse was more concentrated at the end of the pulse as compared to the dusk pulse, any difference in phase shift size could be interpreted as a CT effect rather than a light profile effect. To minimize CT effects, we used pulses of a relatively short duration (as compared to a natural twilight or to the study of Boulos et al.).

We could program the simulator so that different maximum light intensities could be attained. During the entrainment phase of our "high-level" experiments, light intensity gradually reached 250 lux at the end of dawn (Fig. 1) and remained steady at that level until the onset of dusk, when it started to decrease gradually in a mirror-image fashion. In our "low-level" experiments, light intensity reached 150 lux at the end of dawn (Fig. 1) and was maintained at that level until the onset of dusk. The light pulses that were eventually delivered in those experiments were of the same maximum intensity as that reached during entrainment. These light intensities (250 and 150 lux) are higher than the 10 lux used by Boulos et al. (6), but lower than the 450 lux that is normally mea-
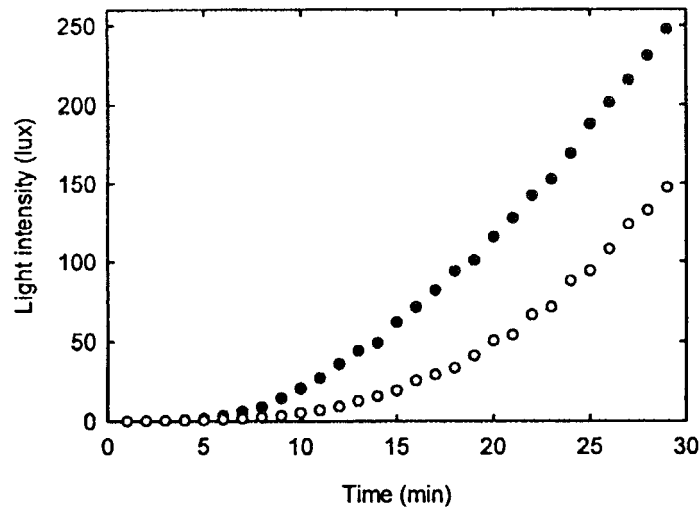
Figure 1. Light-intensity profiles of dawn used in the experiments, as measured at cage level with a Gossen Lunasix 3 photometer with the integrating sphere on. Dusk was the mirror image. Within an experiment, the same profile was used for both dawn and dusk during entrainment, as well as the dawnlike and dusklike pulses during testing. Depending on the experiment, the profile could be of high (upper curve) or low (lower curve) maximum light intensity.

sured in the open in nature when the entire disk of the sun is just below the horizon (9).

Methods

Each experiment involved 30 hamsters, randomly separated between two rooms. After 14–21 days in light-dark, hamsters were exposed, soon after dusk, to a 30-minute light pulse that reproduced either the dawn (one room) or the dusk (the other room) they had been accustomed to during entrainment. In the case of the dawn pulse, light intensity increased gradually from complete darkness to its maximum intensity in 29 minutes (Fig. 1) and then went back to complete darkness within 1 minute. In the case of the dusk pulse, light intensity first went to its maximum within 1 minute and then decreased gradually to complete darkness in 29 minutes. Animals then remained in complete darkness (DD) for the next 10 days. This procedure corresponds to Aschoff's type II method for measuring phase shifts (8). Its main advantage, among others, is to allow the delivery of light pulses at a precise circadian time (or more precisely, zeitgeber time, ZT) for many animals simultaneously (9).

Following the 10 days in DD, animals were reentrained to light-dark as before for a minimum of 14 days, after which they received another light pulse, but this time the 15 animals that had received a dawnlike pulse got a dusklike

one, and vice-versa. This protocol allowed a within-individual paired comparison for phase shift size between dawnlike and dusklike pulses, with a crossover design that controlled for order effect.

Four experiments were conducted as described above, each with new hamsters, representing the combination of two light pulse intensities (250 and 150 lux) and two zeitgeber times at which the pulses were started (ZT13.5, 1.5h after the start of the preceding dusk or, in other words, 1h after its end; and ZT14.5). Two more experiments were added to these, one with the high light intensity and one with the low, but with pulses given at ZT20 (8h after the start of the preceding dusk). In this case, because the pulses were given in the second half of the subjective night, phase advances were obtained.

The six experiments were conducted in the following order: ZT20, high intensity in August–September 1999; ZT14.5, high intensity in November–December 1999; ZT13.5, low intensity in January–February 2000; ZT20, low intensity in March–April 2000; ZT14.5, low intensity in May–June 2000; ZT13.5, high intensity in July–August 2000.

Measurement of Phase Shifts

Wheel rotations were tallied by computer in blocks of 6 minutes with the Dataquest III program (obtained from Mini-Mitter Co., Sunriver, OR). These data were downloaded to another computer, and actograms were graphed with the Tau program (Mini-Mitter Co.). For each pulse given, a regression line was fitted mathematically through the seven daily activity onsets that immediately preceded the pulse, and another was fitted through seven onsets that followed it. (In the case of phase delays, no transients were observed; therefore, we used the seven daily onsets that immediately followed the pulse. In the case of phase advances, a few transients were observed; therefore, we did not consider the first two daily onsets postpulse, only the seven that followed those first two.) The pre- and postpulse regressions were extrapolated onto the day that followed the pulse, and the difference between the two gave the magnitude of the phase shift.

Statistical Analyses

Statistical tests were run with SPSS 7.5 for Windows (SPSS, Chicago). Level of significance was set at $P = .05$. In the first analysis, we used a two-tailed paired t test to compare the effect of dawnlike and dusklike pulses in each of the six experiments (each combination of ZT and light intensity). However, given that a specific prediction could be made a priori regarding the direction of the difference (dusk pulses should lead to greater phase delays than dawn pulses, and dawn pulses should yield larger advances), we also looked at one-tailed P values. Power analysis for the paired t tests followed the method of Zar (10).

We also used an unpaired t test to compare the effect of dawnlike and dusklike pulses for the first half only of each experiment. This approach decreases power ($n = 15$ instead of 30), but it also eliminates any potential order effects that might have added variability to the data (although we did not notice any such effects).

We also looked at the results in a more global way, entering the data of all six experiments into the same three-way analysis of variance (ANOVA). The three factors were pulse type (dawnlike or dusklike), pulse intensity (150 or 250 lux), and the time of pulse delivery (ZT13.5, ZT14.5, or ZT20). In addition to confirming the outcome of the t tests, this approach allowed ancillary insight into the effect of pulse intensity and ZT. Power analysis for the ANOVA followed the method of Zar (10).

RESULTS

Figure 2 shows the average magnitude of the phase shifts obtained in each experiment. In no experiment did a two-tailed paired t test detect a significant
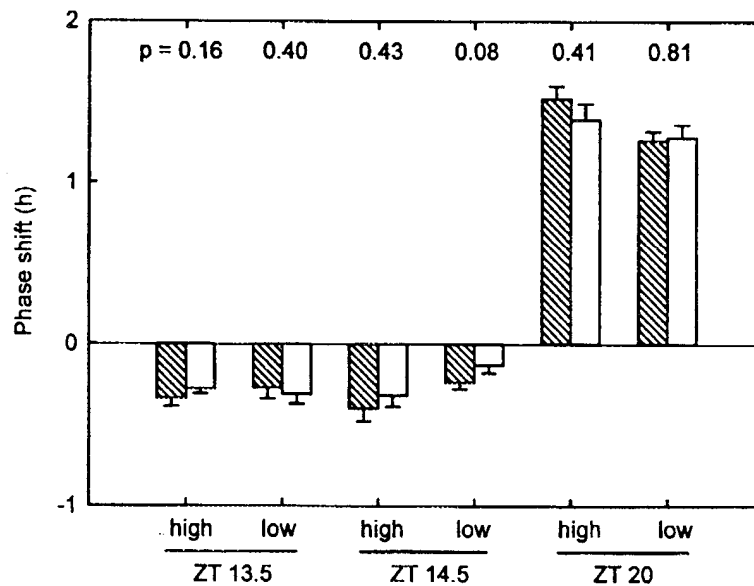

Figure 2. Magnitude of phase shifts (mean ± standard error, $n = 30$) induced by dusklike (hatched bars) and dawnlike (open bars) 30-minute light pulses. Positive and negative values are advances and delays, respectively. High and low refer to the maximum light intensity of the pulses (250 or 150 lux). ZT refers to the zeitgeber time when the 30-minute pulses started. P values from a two-tailed paired t test are indicated for each experiment.

difference between dawn and dusk pulses in the size of the phase shifts they induced. With the relatively large sample size we used ($n = 30$), statistical power was reasonably good. Depending on the experiment (i.e., the variance in the data), the minimum difference detectable 90% of the time ranged between 0.15h and 0.29h.

One-tailed P values were half the two-tailed ones. Analysis with one-tailed paired t tests, therefore, revealed a significant difference ($P = .04$) for the low-intensity pulses given at ZT14.5. There was also a nearly significant difference ($P = .08$) for the high-intensity pulses given at ZT13.5. Both of these differences were in the expected direction, that of a larger phase delay on the part of the dusklike pulses. The other four experiments yielded no significant results with one-tailed paired t tests.

In none of the six experiments did unpaired t tests lead to the detection of a significant difference between dawnlike and dusklike pulses when only the first half of each experiment was considered. This held true for two-tailed, as well as one-tailed, tests ($P = .17-.87$ for two-tailed tests).

The three-way ANOVA confirmed a lack of pulse type effect ($F = 0.183$, $P = .669$). In fact, the only significant results from this ANOVA were a significant ZT effect ($F = 799.9$, $P < .0001$, simply reflecting the fact that advances occurred at ZT20, while delays occurred at ZT13.5 and ZT14.5), and there was a significant interaction term for ZT by pulse type ($F = 7.484$, $P = .001$). The last reflects the fact that high-intensity pulses yielded larger delays (more negative shifts) than low-intensity pulses at ZT14.5 and larger advances (more positive shifts) than low-intensity pulses at ZT20 (Fig. 2). Power for the ANOVA was good as the minimum difference detectable 90% of the time was only 0.13h.

DISCUSSION

Despite the use of various powerful tests (paired t test, ANOVA), good sample size ($n = 30$), two different light intensities, and two different early night ZTs, we failed to detect a significant difference between dawnlike and dusklike pulses in the size of the phase delays they caused, at least with two-tailed statistical tests. Even with one-tailed tests, only one experiment yielded the expected significant result. This is not strong evidence for a differential phase-shifting effect of dawnlike versus dusklike pulses. Thus, our results are similar to those of Boulos et al. (6), who also did not detect any difference between dawnlike and dusklike pulses in the size of the phase delays obtained in hamsters. Despite the fact that our methodology differed from that of Boulos et al. (6), our conclusion and theirs are the same, at least for phase delays.

It could be argued that, despite our use of relatively short pulses, CT effects may have masked any possible promoting effect of dusklike pulses on phase delays. Perhaps dusklike pulses do promote phase delays; however, because the

bulk of the light they delivered fell on a slightly earlier portion of the light PRC (as compared to dawnlike pulses starting at the same time), a time during the early night that corresponds to small delays, they were less effective than dawn-like pulses. If this is the case, it means that we tested our original working hypothesis in a conservative way. But, it also suggests that, if a promoting effect for phase delays does exist, it is likely to be a small one.

Can the apparent absence of potential for larger phase delays be reconciled with the facilitated entrainment observed under long light-dark cycles when a twilight is included (5)? It can if one remembers that in the original entrainment study (5), the periodicity of the light-dark cycle was successively and progressively increased in the same animals, and that greater aftereffects were observed when light-dark cycles included twilights (the free-running rhythms of animals emerging from a long light-dark cycle with twilights were longer than when the light-dark cycles did not include twilights). So, instead of increasing the potential for large phase shifts, twilights may simply decrease the need for large shifts by lengthening the endogenous periodicity of the clock and bringing it closer to that of the zeitgeber.

Although we tested only one time (ZT20) in the second half of the night, we failed again to observe a difference between the effects of dawnlike pulses versus dusklike pulses. In the study of Boulos et al. (6), a 2h bin centered on CT19 was the only time on the PRC when a significant difference between dawn-like and dusklike pulses was observed. Contrary to what might be intuitively expected (given that dawn is a more natural occurrence at the end of the night), dawnlike pulses yielded smaller phase advances than dusklike pulses.

Before we tested for phase shifts, we entrained our hamsters with a light-dark cycle that already included twilight periods. To us, this represented a more "natural" situation as hamsters in nature would theoretically have the chance to witness twilight every day. This also represented a better counterpart to classical experiments in which rectangular pulses had been given to animals that had been entrained with a rectangular light-dark cycle. This being said, we do not know if hamsters in nature are indeed exposed to the full twilight. In one laboratory experiment, hamsters that were provided with a dark nest box emerged from the box before dusk and were therefore exposed to twilight, but this was with a light-dark cycle having a maximum light intensity of only 10 lux (11). Also in a laboratory setting and with access to a dark nest box, flying squirrels, Glaucomys volans, sampled the light repeatedly, but only for a few seconds or minutes at a time before lights off (12). So, the possibility remains that nocturnal animals that sleep in burrows or tree holes may seldom be exposed to full twilight— and the same may even apply to some diurnal fossorial rodents (13). Diurnal animals that sleep in more open areas (birds that roost in trees, for example) may hold better promise for study in that regard as such animals are usually exposed to the complete gradual change in light intensity that takes place at dawn and dusk.

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