Resetting of the hamster circadian system by dark pulses

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Canal, M. M., H. D. Piggins. Resetting of the hamster circadian system by dark pulses. *Am J Physiol Regul Integr Comp Physiol* 290: R785–R792, 2006. First published October 20, 2005; doi:10.1152/ajpregu.00548.2005.—Circadian rhythms of animals are reset by exposure to light as well as dark; however, although the parameters of photic entrainment are well characterized, the phase-shifting actions of dark pulses are poorly understood. Here, we determined the tonic and phasic effects of short (0.25 h), moderate (3 h), and long (6–9 h) duration dark pulses on the wheel-running rhythms of hamsters in constant light. Moderate- and long-duration dark pulses phase dependently reset behavioral rhythms, and the magnitude of these phase shifts increased as a function of the duration of the dark pulse. In contrast, the 0.25-h dark pulses failed to evoke consistent effects at any circadian phase tested. Interestingly, moderate- and long-dark pulses elevated locomotor activity (wheel-running) on the day of treatment. This induced wheel-running was highly correlated with phase shift magnitude when the pulse was given during the subjective day. This, together with the finding that animals pulse during the subjective day are behaviorally active throughout the pulse, suggests that both locomotor activity and behavioral activation play an important role in the phase-resetting actions of dark pulses. We also found that the robustness of the wheel-running rhythm was weakened, and the amount of wheel-running decreased on the days after exposure to dark pulses; these effects were dependent on pulse duration. In summary, similarly to light, the resetting actions of dark pulses are dependent on both circadian phase and stimulus duration. However, dark pulses appear more complex stimuli, with both photic and nonphotic resetting properties.

Phase shift; nonphotic; wheel-running; entrainment

**MATERIALS AND METHODS**

A total of 113 adult (90–110 g) male LVG 1AK Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River (Margate, Kent, UK). Groups of up to 20 hamsters were initially group-housed for 1–2 wk under a 14:10-h light-dark cycle and then placed in individual cages (40.5 × 24 × 12 cm) equipped with stainless steel

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running wheels (16 cm diameter). In all instances, water and food (Rat and Mouse Expanded Diet, B&K Universal, Hull, UK) were available ad libitum. Each cage was mounted on a stainless-steel platform above a plastic litter tray. This tray was removed for cleaning twice a week at random times during the circadian cycle. Previous observations revealed that this cleaning procedure did not shift hamster rhythms. After spending a further 2 wk under the light-dark cycle, the room lights were left on such that the hamsters were now in constant light (LL, ∼200 lux at cage floor level). The animals free-ran for at least 2 wk in LL to establish robust free-running rhythms before dark pulse treatment. Four different duration dark pulses were given: 0.25 h (n = 59 data points), 3 h (n = 72), 6 h (n = 96), and 9 h dark pulse (n = 52). In all groups, the dark pulse treatment consisted of switching the room lights off for varying durations. To avoid exposure to other nonphotic stimuli, the animals were not disturbed in any other way (e.g., noise, cage change, room change) during the dark pulse treatment. The hamsters free-ran an average of 45 days in LL and were randomly assigned a minimum of two dark pulses, separated by at least 10 days. All procedures were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986.

For each animal, the number of wheel revolutions was recorded every 10 min throughout the experiment using the Chronobiology Kit (Stanford Software Systems, Santa Cruz, CA). For visualization of the activity rhythms, the data were double-plotted offline as actograms. The magnitudes of the phase shifts produced by the dark pulses were assessed manually using the line of best fit method (28) by three different experienced researchers who were unaware of the circadian phase or duration of the pulse. Briefly, a line was drawn through the onset of activity during the 10 days before the pulse and extrapolated to predict an onset time on the day following pulse treatment. A second line was drawn through the onsets of activity on the 10 days after pulse (discounting transient responses on the first day or two following the dark pulse) and interpolated to predict an onset on the day after the pulse treatment. These were then subtracted to yield a phase shift. The value for each phase shift was calculated by averaging the individual ratings of the three researchers. By convention, circadian time 12 (CT 12) was defined as the onset of the running wheel activity rhythm. For each pulse duration, measures of the shifts were grouped into 4-h bins, so that each CT bin contained data from a minimum of six animals with each animal contributing only one data point per pulse duration per CT bin.

The chronobiology software package El Temps (A. Díez-Noguer, Universitat de Barcelona, Barcelona, Spain) was used to calculate several variables of the motor activity rhythm. The period of the free-running locomotor activity rhythm (Tau) was calculated before and after the pulse, since it can be a more precise and objective method of assessing the shifting effects of nonphotic stimuli than eye-fitting regression lines (7). Tau was estimated by $\chi^2$-periodogram (33). The percentage of variance explained (PVE) by the highest peak obtained in the periodogram was used as an indicator of the strength of the circadian rhythm in the motor activity pattern. Using the mean waveform of intensity of motor activity, we also calculated the duration of the alpha or active phase (ta) and the intensity of activity performed during the alpha phase (Aa). To establish whether dark pulses alter these characteristics of the circadian rhythm, we subtracted averages of these variables (Tau, PVE, ta, and Aa) on the 10 days before the pulse from the averages on the 10 days after the pulse, thus obtaining the following: diftau, difPVE, difta, and difAa. To discriminate the intensity (wheel-running revolutions per minute) of motor activity induced by the dark pulse from the typical intensity of daily motor activity, we subtracted the average mean waveform for the 10 days before the pulse from the waveform on the day of the pulse. The result is the change in intensity of motor activity induced by the pulse (MIP).

Because awake, active hamsters do not necessarily engage in wheel-running during a dark pulse and because behavior during the pulse influences the characteristics of the subsequent phase shift, we assessed the behavior of nine animals that received a 3-h dark pulse during CT 4–8. With the aid of infrared goggles, we rated their behavior for 10 s every 7 min. We classified the hamsters’ behavior into four categories: active (running in the wheel or moving around the cage), eating/drinking, resting, or asleep.

For the statistical assessment, a total of five general linear models were performed using Systat ver. 10 (SPSS, Chicago, IL). In the first model, a general analysis of all of the data was carried out. Here, the independent variables were pulse duration and CT of the onset of the dark pulse. The dependent variables were phase shift, diftau, difPVE, difta, difAa, and MIP. When a statistically significant difference was encountered, a subsequent ANOVA with a Bonferroni post hoc test was applied. Because pulse duration is a quantitative variable, the ANOVA assessed linear regression. In addition, four general linear models were assessed to test, for each pulse duration (0.25, 3, 6, and 9 h), the effect of the CT on key variables of the free-running rhythm. In these models, the independent variable was CT, and the dependent variables were the same as in the general model. When examining correlative relationships between variables, a simple Pearson correlation was calculated. Significance was set at $P < 0.05$ for all statistical tests.

RESULTS

The resetting actions of dark pulses on hamster behavioral rhythms in LL were dependent on both circadian phase and duration of the stimulus (Fig. 1, A–C). The CT, at which a dark pulse began, significantly influenced the magnitude and direction of the subsequent phase shift ($F_{5,243} = 11.374, P < 0.001$). As shown in Fig. 2, dark pulses produced phase delays in the late subjective night (with a maximal delay at CT 20–24) and phase advances during the subjective day (maximal advance at CT 4–8). We also found a significant ($F_{3,243} = 2.778, P < 0.05$) relationship between the duration of a dark pulse and phase shift magnitude, indicating that as the duration of the pulse lengthened, the size of the phase shift increased (Fig. 2). The 0.25-h dark pulses typically produced the smallest average phase shifts (mean absolute value ± SD = 0.27 ± 0.34 h), while the 9-h-dark pulses elicited the largest average phase shifts (mean absolute value = 2.56 ± 2.18 h).

Because dark pulse duration had a highly significant effect on the magnitude of the phase shifts, we plotted PRCs for each pulse duration and further assessed the effect of CT in influencing phase shifts. We found a statistically significant effect of CT on the magnitude and direction of the phase shifts evoked by the 3 h ($F_{5,50} = 6.564, P < 0.001$), 6 h ($F_{5,71} = 10.995, P < 0.001$), and 9 h ($F_{4.45} = 3.538, P < 0.01$) dark pulses, but not the 0.25 h ($P > 0.05$) dark pulse (Fig. 3, A–D). This indicates that these actions of 0.25-h dark pulses on the wheel-running rhythm did not vary across the circadian times studied. For example, at CT 4–8, there was an average shift of 0.15 ± 0.38 h, while at CT 20–24, the average shift was 0.17 ± 0.46 h (Fig. 3A).

The 3-h dark pulses (Fig. 3B) produced phase advances during the subjective day (maximum of 2.32 ± 2.48 h at CT 4–8). Phase delays were confined between CT 0 and CT 4 (~0.91 ± 1.24 h). The mean phase shift between CT 16 and CT 24 (0.24 ± 0.61 h) was very small, implying a dead zone, or time of the circadian cycle, when a stimulus fails to significantly reset the onset of the locomotor rhythm. Although the population of animals tended to respond in a similar way, some individuals produced large phase shifts (e.g., 10-h phase advance at CT 6, and 3.7 h phase delay at CT 1).
The shape of the 6-h dark pulse PRC (Fig. 3C) was similar to that of the 3-h dark pulse PRC, with the phase-advance zone occurring during midsubjective day (maximum of $2.9 \pm 2.05$ h at CT 4–8). However, compared with the 3-h dark pulse PRC, there was an extended phase delay zone, ranging from CT 16 to CT 4 (maximum of $-1.3 \pm 2.6$ h at CT 0–4). The dead zone occurred earlier and for a smaller proportion of the circadian cycle (CT 12–16) than for the 3-h dark pulse PRC.

The 9-h dark pulses (Fig. 3D) also produced phase advances during the subjective day (maximum of $4.22 \pm 2.89$ h at CT 4–8). The phase-delay portion of the curve was mainly located between CT 16 and CT 24 (maximum of $-2.96 \pm 1.47$ h at CT 20–24). Phase shifts to dark pulses beginning CT 0–4 were highly variable; three animals pulsed between CT 0 and CT 2 showed large phase delays ($-3.75 \pm 1.39$ h), and another three hamsters pulsed between CT 2 and CT 4 showed large phase advances ($6.70 \pm 3.86$ h). Large-magnitude shifts to 9-h dark pulses were also seen at other CTs. For example, one hamster produced a phase advance of $\sim 11$ h after a 9-h dark pulse at CT 7, whereas another animal phase-delayed by approximately $-4.6$ h in response to a dark pulse at CT 0. Similar to the 6-h dark pulse, no consistent resetting effects were observed when pulses were given at CT 12–16, indicating conservation of the dead zone at this early phase of the subjective night.

To evaluate the effect of pulse-induced motor activity on the magnitude of the phase shift, we subtracted the average intensity of daily activity during the 10 days before the dark pulse from the intensity of motor activity on the day of the pulse. Results show that in most cases, the intensity of motor activity performed by the animal was augmented on the day of the pulse compared with the 10 previous days (the average increase in wheel-running activity evoked by dark pulses was $1.6 \pm 4.5$ rpm, indicating that the hamsters ran an additional 1.16 km on the day they were dark-pulsed). This increase in the motor activity levels occurred at various points throughout the duration of the pulse. The pulse-induced motor activity was
independent of the CT at which the dark pulse was given ($P > 0.05$), but was highly dependent of the duration of the dark pulse ($F_{3,243} = 7.753, P < 0.001$); longer-duration dark pulses induced higher levels of motor activity on the day of the pulse (9-h dark pulse average = $3.5 \pm 5.2$ rpm or $2.5$ km) (Fig. 4).

Statistically significant correlations between MIP and magnitude of phase shift were found at CT 0 – 4, 4 – 8, and 8 – 12 ($P < 0.05$), but not for nighttime CT bins. This indicates that during the subjective day, the animals that ran more intensely in the wheel as a consequence of the pulse were also more likely to show large phase shifts.

In addition, we examined the general behavior of nine hamsters during a 3-h dark pulse beginning CT 4 – 8; a phase of the circadian cycle when this stimulus evokes significant phase advances (see above). These animals advanced an average of $2.22 \pm 2.11$ h. The dark pulse aroused the animals as they spent more time running in the wheel, moving around the cage, or feeding (55.8% ratings) than resting or sleeping (44.2%) (Fig. 5).

To determine whether the dark pulses altered the robustness of the wheel-running rhythm, we evaluated whether either the time of pulse onset or pulse duration altered difPVE. The CT at which the hamsters were dark-pulsed did not affect the strength of the circadian rhythm of motor activity after the pulse ($P > 0.05$). However, the strength of the rhythm did vary depending on the duration of the dark pulse ($F_{3,243} = 7.988, P < 0.001$). The subsequent quantitative analysis of variance indicated a highly significant linear regression ($F_{1,273} = 31.461, P < 0.001$), such that the locomotor activity rhythm became progressively weaker the longer the duration of the dark pulses (Fig. 6A).

Fig. 2. Phase-shifting effects to dark pulses are circadian phase and pulse duration dependent. Histograms representing the average of phase shift for each pulse duration at each CT bin (means ± SE).

Fig. 3. Scatterplot phase response curve (PRCs) for each pulse duration: 0.25-h dark pulse (A), 3-h dark pulse (B), 6-h dark pulse (C), and 9-h dark pulse (D). Each dot represents a phase shift of an individual animal to a specific dark pulse. Note the absence of consistent phase shifts for the 0.25-h dark pulse. Longer-duration dark pulses (3, 6, and 9 h) caused robust phase advances; however, the delay portion was more variable. Note also the gradual transition between advances and delays, as the pulse duration lengthens. The variability of the shifts to 9-h dark pulses at CT 0–4 in Fig. 2 is due to the sharp transition between phase delays and advances at CT 2 (D).

Fig. 4. Dark pulses increase activity on the day of the treatment. Average motor activity induced by the dark pulse (MIP) for each pulse duration (means ± SE; see MATERIALS AND METHODS for further explanation). Rpm, revolutions per minute.
loss of circadian rhythmicity, change in duration of the alpha phase or change in the intensity of activity performed during the alpha phase. These alterations in the rhythm also occurred spontaneously (Fig. 1D), but in most instances, they appeared subsequent to dark pulsing. Collectively, they were infrequent and were only observed in 41 out of 331 dark-pulse responses (Fig. 7). Dark pulses also caused Tau lengthening and shortening (Fig. 1B), but unlike phase shifts, these changes did not vary by CT or pulse duration ($P > 0.05$, data not shown).

**DISCUSSION**

This is the first study to systematically evaluate the transient and longer-lasting effects of varying durations of dark pulses on circadian rhythms in hamsters. The results reveal that short duration pulses (0.25 h) do not consistently alter the locomotor rhythms of hamsters free-running in LL, whereas longer 3–9 h pulses elicit large-amplitude advances when given during the middle of the subjective day and large-amplitude delays when given during the late subjective night/early subjective day. Inspection of the PRCs illustrates that with the 3–9 h duration of dark pulses, there is a phase in subjective night at which the pulses do not have any significant mean phase-resetting effects, indicating a dead zone of the circadian pacemaker’s response to these stimuli. In addition, dark pulses induce an increase in the intensity of wheel-running on the day of the pulse. Further, the intensity of locomotor activity during the pulse correlates with the magnitude of the phase shift only when the dark treatment occurs during the subjective day, a time when nocturnal animals are usually resting. Indeed, direct observation of hamsters dark-pulsed during the subjective day showed that animals were behaviorally active for most of the 3-h pulse at this phase. Here, we have also determined that dark pulses have other longer lasting influences that have not been quantified in other studies. After long-duration dark pulses, the locomotor activity rhythm became weaker in intensity (Aa, rpm) and in strength (PVE). Therefore, in addition to transiently promoting locomotor activity and phase-shifting the circadian pacemaker, dark pulses can also exert various longer-lasting effects on clock-driven behavioral rhythms.

In contrast to previously published work (32), we did not find consistent phase shifts to 0.25 h dark pulses. Rosenwasser and Dwyer (32) report that 0.25-h dark pulses evoked advances (~0.6–0.7 h) in hamsters free-running in LL when given at CT 11.5 or CT 12, whereas we found that this stimulus had negligible effects at this or any other phase of the circadian cycle. This discrepancy is likely attributable to differences in
experimental methodologies. In our study, animals were always housed in the same room, and dark pulses were given by switching the lights of the room off and on. In contrast, Rosenwasser and Dwyer (32) moved the animals in their investigations to a different room for the duration of the pulse. It is possible, therefore, that their hamsters were subjected to at least three stimuli (dark pulse, novel environment, and mechanical arousal) whose interactions yielded larger shifts. The intensity of room lighting is unlikely to be a main factor in accounting for this discrepancy; we used a higher light intensity (≈200 lux) than the study of Rosenwasser and Dwyer (≈100 lux), and higher levels of background illumination reportedly increase the magnitude of phase shifts to dark pulses at this late subjective day phase rather than negating it (13, 22). Although the size of the running wheel has also been shown to have an effect on the magnitude of phase shifts (11), the diameter of the wheels in this (16 cm) and in the Rosenwasser and Dwyer study (17.5 cm) (32) was very similar. Therefore, differences in the experimental protocols probably underpin the discrepancy in the results of these two studies.

Our PRC for the 3-h dark pulse is in general agreement with the previous findings of other laboratories that used 2- or 3-h dark pulses (5, 16, 23, 29, 30). We observed slightly larger phase advances, which occurred slightly earlier (CT 4–8) than in the above-mentioned studies (CT 9–12). The lower background illumination used in the previous studies, compared to our investigation, may account for some of these small differences. In contrast, the phase-delaying actions of 3-h dark pulses are more variable. Ellis et al. (16) found delay responses to 3-h dark pulses (between CT 17–23) to be inconsistent (only 4 animals out of 22 phase-delayed). Boulos and Rusak (5) observed ~1-h phase delays to 2-h dark pulses starting during the late subjective night-early subjective day. These shifts are similar in magnitude to the phase delays in our study, although here, delays were only evoked during the early subjective day. Taken together, it is clear that dark pulses of 2–3 h duration robustly phase-advance hamster locomotor rhythms around the middle to late subjective day but have variable phase-delaying actions when given during the late subjective night/early subjective day. Hence, longer-duration dark pulses appear to be necessary to evoke consistent phase delays in the hamster wheel-running rhythm (see below).

The PRC that we obtained to 6-h dark pulses is similar in shape to the 6-h dark pulse PRC of Boulos and Rusak (5) and comparable to the PRC of Harrington and Rusak (17). The maximal phase advances compare favorably in ours (~2–3 h) and those of Rusak and colleagues (5, 17) and are slightly larger than the ~1.8-h phase advances to 6-h dark pulses given at CT 6 in the study of van Reeth and Turek (36). However, as is the case for the 3-h dark pulses, the phase-delaying effects of the 6-h pulses during the late subjective night/early subjective day vary across the different studies. For example, we observed delays of ~1–1.3 h evoked by 6-h pulses at CT 20–24, which are similar to the delays reported by Harrington and Rusak (17), but considerably smaller than the phase-delaying actions of 6-h dark pulses (~2–3 h) given at comparable circadian phases in the study of Boulos and Rusak (5). Further, van Reeth and Turek (36) found only small phase delays (~0.6 h) to 6-h pulses given at CT 18. Although background illumination levels vary across these different investigations ~80 lux in Boulos and Rusak (5), ~160 lux in Harrington and Rusak (17), ~200 lux in our study, and 600 lux in van Reeth and Turek (36), there is no obvious pattern in the differences of the phase shifts in these studies, suggesting that other uncharacterized experimental conditions influence the magnitude of phase delays to the 6-h dark pulses.

The PRC to the 9-h dark pulses is the first to be reported. The shape of the PRC shares many characteristics with our 6-h PRC, as well as the PRCs to 6-h dark pulses from other laboratories (5, 17). The main difference is that, except for the dead zone, where no significant mean phase shifts were found for any pulse duration, the magnitudes of the phase shifts evoked by this 9-h stimulus are considerably larger than shorter-duration dark pulses. In contrast to the 3-h PRC, and similarly to the 6-h PRC, the 9-h PRC has a clear phase-delaying zone that occurs between CT 20 and CT 2. Similar to the 6-h dark PRC, the 9-h PRC has a clear phase-delaying zone that occurs between CT 20 and CT 2. Similar to the 6-h dark PRC, the transition from phase-advancing to phase-delaying actions of the 9-h pulses was gradual, with an apparent ~4-h dead zone around early subjective night. Thus, as the duration of the dark pulse increases, the transition between phase advances and phase delays becomes gradual, so that the dead zone is progressively reduced. This has been previously found in the dark pulse PRC (5), and is attributable to the use of relatively long pulses. It is also worth noting that the transition from phase delays to phase advances evoked by 9-h pulses is very abrupt, sharing some features with type 0 circadian phase-resetting (39). This is in accordance with the observations that it is possible to change from type 1 to type 0 resetting by increasing key parameters of a stimulus such as its length (9, 39).

Given the long duration of some of our dark-pulse treatments, it is possible that transitions from light to dark (LD) and from dark to light (DL) contribute to the phase shifts to these pulses, since exposure of Syrian hamsters to sudden LD and DL transitions, phase-dependently shift free-running rhythms (1). Our individual 3-h, 6-h, and 9-h PRCs were plotted according to the CT at onset of the dark pulse, and although the offsets of the pulses (DL transition) must occur at different CTs due to the different pulse durations, the shapes of the three curves are remarkably similar. Further, at CT 0, there are phase delays in the 3-h, 6-h, and 9-h dark pulse PRCs, which cannot be explained by the addition of the shifts that can be evoked by LD and DL transitions (the magnitude of the shifts extrapolated from Albers (1) do not predict the phase shifts seen here). It is also notable that Mistlberger and colleagues (23) found that LD and DL transitions do not significantly influence phase advances to 3-h dark pulses given at CT 6. These observations strongly suggest that the light and dark transitions alone are not sufficient to predict and/or account for the effect of dark pulses on the phase of the circadian rhythm.

Here, for the first time, we also analyzed the effects of dark pulses on a range of variables characterizing the motor activity rhythm of the animal. We observed that the duration of the dark pulse is not only a key factor in determining the magnitude of the phase shift, but also plays an important role on the characteristics of the locomotor rhythm after the pulse. With long pulses, animals run less during the 10 days after the pulse and behavioral rhythms weaken. The level of activity is believed to have a strengthening feedback effect on the circadian clock (35); thus, a lower level of activity may explain the reduced robustness of the rhythm. This weakening of the rhythm, though, does not seem to account for the splitting, loss
of circadian periodicity, and other alterations observed in some animals as a consequence of exposure to a dark pulse, since the incidence of these alterations is similar for all dark-pulse durations. Nevertheless, the number of animals that split was very low compared with similar studies, probably because our animals remained in LL for a relatively short period (an average of 45 days), compared with the 90–360 days of the previously mentioned studies (5, 16, 17).

The CT or time when the dark pulse is given is critical in determining the direction of the shift in the phase of the circadian rhythm. Curiously, it is not an important factor in determining how the dark pulse subsequently alters some defining characteristics of the wheel-running rhythm (e.g., strength, duration, and intensity of activity during alpha phase). This suggests that the circadian pacemaker and the behavioral rhythms that it controls are sensitive to these actions of dark pulses at all circadian phases. Changes in Tau were also observed after many dark pulses: some phase delays are accompanied by a Tau increase, whereas some phase advances accompany Tau shortening, similar to the alterations reported by Boulos and Rusak (5). However, the overall pattern was not phase dependent, because the changes in Tau were generally small (mean change in Tau of ~5 min).

Both forced exercise and novelty-induced wheel running have been repeatedly shown to evoke a nonphotic pattern of phase-resetting in rodent locomotor rhythms (30, 38). Simple behavioral arousal also resets rodent rhythms in a phase-dependent pattern (30). Interestingly, physical restraint attenuates or completely blocks the phase-advancing actions of dark pulses given during the subjective day (13, 29, 32, 36). On this basis, these authors have hypothesized that wheel-running activity is necessary for dark pulses to reset the pacemaker. In previous studies, only the wheel-running activity exerted by the animal during the pulse (calculated as total number of revolutions) has been taken into account when studying the effects of locomotor activity on the phase-shifting effects of dark pulses (8, 12, 32, 37), and this has yielded contradictory results. This method has the disadvantage of considering the total amount of motor activity as a whole; it does not distinguish between usual and pulse-induced motor activity. Here, by subtracting the average motor activity of the days previous to the pulse, from the motor activity of the day of the pulse, we are able to differentiate between the normal activity of the animal and the activity uniquely related to the dark treatment (MIP). The study of this new variable showed that dark pulses generally induce an increase of the wheel-running intensity on the day of the pulse and that there is a significant correlation between the pulse-induced activity and the magnitude of the phase shifts. Further, the average revolutions per minute induced by the pulse were only correlated to the magnitude of the phase shift in the pulses starting during the subjective day, a time when the hamster normally rests. Therefore, although dark pulses evoke an increase of motor activity in all circadian phases, this induced activity is only a key factor in phase resetting during the subjective day, as locomotor activity displayed at times when the animal is active per se fail to produce consistent phase shifts. This is in agreement with a study of Dwyer and Rosenwasser (13), in which they also found that dark-pulse-induced phase shifting during early subjective night was not mediated by activity levels.

In addition, our behavioral ratings of hamsters during a 3-h dark pulse given at CT 4–8 (subjective day), indicate that the animals were active during most of the dark pulse. This suggests that it is important that the animal is aroused and activated by the dark pulse in order for this stimulus to produce a large phase shift. This is in broad agreement with recent findings showing that behavioral arousal as a consequence of sleep deprivation during a dark pulse accounts for phase shift size (2, 23). Overall, these results support the idea that a combination of behavioral activation, together with some degree of locomotor activity, plays an important role in the phase-resetting properties of dark pulses. Further studies using infrared motion detectors and/or implanted telemetric probes are required to test this idea.

It is notable that, in the 3-h dark pulse PRC, phase advances are more readily evoked than are phase delays; a pattern of resetting very similar to that induced by nonphotic stimuli. By contrast, longer pulses of dark (6–9 h) phase-delay behavioral rhythms when given during the late subjective night/early subjective day, actions very different to those of many nonphotic stimuli. This suggests that longer pulses cannot be categorized simply as nonphotic. Consistent with this view, lesions of brain structures that convey nonphotic information to the hamster SCN do not block phase advances to 6-h dark pulses given during the late subjective day (17, 38). Concordant with this, Rosenwasser and Dwyer (31) recently modeled a dark pulse PRC by combining nonphotic and photic PRCs. Indeed, at the molecular level, both photic and nonphotic events occur in the hamster SCN with 4–6 h of dark pulse exposure (8, 22). Therefore, dark pulses are likely to have both nonphotic and photic properties, the combination of which varies with pulse duration and indicates that dark on a background of light is a complex stimulus.

Here we have shown that like light, the magnitude of phase shifts to dark pulses, depends on the duration of the pulse. We found that the shortest dark pulse capable of producing a significant PRC is 3 h. Because a 2-h dark pulse evokes significant phase shifts (5) and because we have observed that 1-h dark pulses do not (MM Canal and HD Piggins, unpublished observations), it is likely that the threshold duration for a dark pulse to evoke time-dependent phase shifts in the hamster is between 1 and 2 h. In contrast, light pulses on the order of a few seconds to minutes are able to induce effective shifts in the locomotor rhythm and SCN neuronal rhythm (6, 18, 27), confirming that light is a more potent zeitgeber compared with darkness. Nevertheless, when given at the appropriate time and “dose,” dark pulses efficiently shift mammalian circadian rhythms. As diurnal rodents also respond to dark pulses (19), this kind of stimuli may prove useful in the treatment of desynchronization problems in humans such as those suffered by shift workers, the elderly, as well as time-zone travelers.

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