

## Running activity mediates the phase-advancing effects of dark pulses on hamster circadian rhythms

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**Summary.** Pulses of darkness can phase-shift the circadian activity rhythms of hamsters, *Mesocricetus auratus*, kept in constant light. Dark pulses under these conditions alter photic input to the circadian system, but they also commonly trigger wheel-running activity. This paper investigates the contribution of running activity to the phase-shifting effects of dark pulses. A first experiment showed that running activity by itself can phase-shift rhythms in constant light. Hamsters were induced to run by being confined to a novel wheel for 3–5 h. When this was done at circadian times (CT) 0, 6, and 9, the mean steady-state phase-shifts were 0.6 h, 3.5 h, and 2.3 h, respectively. The latter two values are at least as large as those previously obtained with dark pulses of similar durations and circadian phases. A second experiment showed that restricting the activity of hamsters during 3-h dark pulses at CT 9 reduces the amplitude of the phase-shifts. Unrestrained animals phase-advanced by 1.1 h, but this shift was halved in animals whose wheel was locked, and completely abolished in animals confined to nest boxes during the dark pulse. Activity restriction in itself (without dark pulses) had only minimal phase-delaying effects on free-running rhythms when given between ca. CT 10 and CT 13. These results support the idea that, in hamsters at least, dark pulses affect the circadian system mostly by altering behavioural states rather than by altering photic input to the internal clock.

### Introduction

Light is the most important environmental factor affecting the circadian system of vertebrates. In

*Abbreviations:* CT circadian time; DD constant darkness; LD light-dark; LL constant light; PRC phase response curve;  $\tau$  period of rhythm

the laboratory, phase-shifts of activity rhythms are obtained when pulses of light are given to animals during their subjective night, a time when darkness normally prevails. Interestingly, phase-shifts can also be obtained with the reverse approach, i.e. by giving pulses of darkness at times when light is normally present. Experimental protocols in this latter case have included enforcing periods of darkness during the light portion of light-dark (LD) cycles self-selected by the animals (Wahlström 1965), transferring animals from an LD cycle to constant light (LL) and exposing them to a pulse of darkness soon after (Klein et al. 1985), giving pulses of darkness after long exposure to LL (Subbaraj and Chandrashekar 1978; Boulos and Rusak 1982; Ellis et al. 1982), or transferring animals from LL to constant dark (DD) (Subbaraj and Chandrashekar 1981; Albers 1986). Almost all of these studies have compared the phase-shifting effects of darkness with those of light applied in a mirror-image protocol. Implicit in this comparison is the idea that dark pulses, like light pulses, may affect the circadian system by directly altering the photic input to the internal clock.

In at least one species, there are now grounds for questioning this idea (Mrosovsky 1988). Syrian hamsters, *Mesocricetus auratus*, are often aroused and run vigorously in their running wheel during dark pulses given in LL (personal observation; see also Fig. 1 in Boulos and Rusak 1982, and Figs. 5 and 7 in Ellis et al. 1982). Induced wheel-running by itself can phase-shift hamsters' activity rhythms (Reeb and Mrosovsky 1989a). The general shape of the phase response curve (PRC) to pulses of induced wheel-running (Reeb and Mrosovsky 1989a) is similar to that resulting from dark pulses (Boulos and Rusak 1982; Ellis et al. 1982) or from LL-to-DD transitions (Albers 1986). Pulses given in the late subjective day result in phase-advances, pulses in the early subjective day have no effect

or yield phase-delays, and pulses in the subjective night have no consistent effect. A further similarity between dark pulses and wheel-running pulses is that both must last for several hours to produce large phase-shifts (see Lees et al. 1983; Reebbs and Mrosovsky 1989b). In this they greatly differ from light pulses, which are effective even when only 10–15 min long (DeCoursey 1960; Daan and Pittendrigh 1976). Therefore the question arises: Could the phase-shifting effects of dark pulses be caused by running activity during darkness rather than by darkness itself? In this paper we present both indirect and direct evidence that most of the phase-shifting effects of dark pulses do indeed result from the running activity triggered by darkness.

### I. Pulses of induced wheel-running in LL

If running activity is involved in the phase-shifting effects of dark pulses, then running should by itself be sufficient to produce phase-shifts in LL, and shift amplitude should be comparable to that caused by dark pulses. The response of hamsters to pulses of wheel-running has already been investigated in DD (Reebbs and Mrosovsky 1989a) but not systematically in LL (however, see Mrosovsky 1988 for examples of shifts caused in LL by manipulations that often induced wheel-running). Here we gave hamsters 5-h pulses of wheel-running starting at circadian times (CT) 0 and 6 (i.e. the beginning and middle of subjective day, respectively), and 3-h pulses starting at CT 9 (late subjective day).

### Material and methods

Twenty-nine male hamsters (LAK:LVG, from Charles River Quebec) were put into LL when 113 days old. They had previously been exposed to an LD 14:10 cycle that 30 days before had been phase-advanced by 8 h. They were housed in metal-walled cages (36 × 20 × 30 cm) with free access to food, water, and a running wheel (17.5 cm diameter) connected to an Esterline-Angus recorder. Light intensity (Gossen Profisix light-meter) within the cages ranged from about 80 lux in the running wheel to about 30 lux in the shadowy corners where the hamsters slept. Room temperature was about 21 °C.

To give pulses of induced activity, we awoke the hamsters and moved them from the shadowy corners of their home cages to metal-sided wheels (Wahmann Co.), 35 cm in diameter, from which there was no exit. Light intensity inside these wheels was about 20 lux. The hamsters had never been in such wheels before. They ran on their own volition in them, presumably because of arousal caused by the novelty of the situation. They were left undisturbed in the wheels throughout the pulses, except during the 5-h pulses; in the middle of these long pulses, we transferred the hamsters back to their home cages for 10 min to allow them to drink and eat (which they always did). Run-

ning activity in the exercise wheels was recorded on the Esterline-Angus.

In the 5th week of LL, 15 hamsters were given a single 5-h pulse starting at CT 6. Nineteen days later, 11 hamsters (including 2 chosen from the 15 that had previously received a pulse at CT 6) were given a 5-h pulse starting at CT 0. At the same time 8 other hamsters (including 3 chosen from the 15 that had been pulsed before) received a 3-h pulse starting at CT 9.

We mounted actograms by pasting successive 24-h activity records below each other. Phase-shifts were quantified as follows. A regression line was calculated for the 7 daily activity onsets (onsets 1–7) immediately preceding the pulse. A second regression line was calculated for 7 consecutive onsets (10–16) following the pulse. The two daily onsets (8–9) immediately following the pulse were not included in any regression. Two types of phase-shift were then calculated. The immediate phase-shift was defined as the difference between the observed onset 9 and the onset 9 predicted by extrapolation of the pre-pulse regression line. The steady-state phase-shift was calculated as the difference between the extrapolations of both regression lines at onset 8 (the same was done at onset 9, and the results were essentially similar). CTs were calculated with reference to onset 8 (CT 12) as predicted by extrapolation of the pre-pulse regression line. The free-running period ( $\tau$ ) of the rhythms often changed after pulses, and this change was calculated by subtracting the slope of the pre-pulse regression line from the slope of the post-pulse regression line. On 4 occasions (at least one animal in each group) the activity rhythm split (see Pittendrigh and Daan 1976) within 7 days of the pulse being given, and no shift or  $\tau$  change could be calculated.

### Results

All animals ran virtually throughout the pulses. Running in the middle (CT 6) or late (CT 9) subjective day resulted in phase-advances of a few hours, while running at the beginning (CT 0) of the subjective day produced phase-delays of about 1 h (Table 1, Fig. 1). There was no significant difference between immediate and steady-state phase-shifts, except for the 5-h pulses at CT 0 where the steady-state shifts were smaller than the immediate shifts (two-tailed Sign test,  $P < 0.05$ ) (Table 1).  $\tau$  almost always increased after a pulse, but the amount of change was slight (Table 1, Fig. 1).

### Discussion

Although designed to test for the effects of wheel-running activity only, our experimental manipulation also entailed a small change in the photic environment of the hamsters. When transferred from the corners of their cages to the metal-sided exercise wheel, the hamsters experienced a decrease in ambient light intensity from 30 to 20 lux. However, this is probably too small a change to have had a major effect. Swade and Pittendrigh (1967) could not entrain a Syrian hamster to a light cycle whose

**Table 1.** Effects of pulses of novelty-induced running on the activity rhythms of Syrian hamsters in LL. Shown are means  $\pm$  SD, with the range in parentheses. Negative phase-shift values indicate delays, while positive values indicate advances

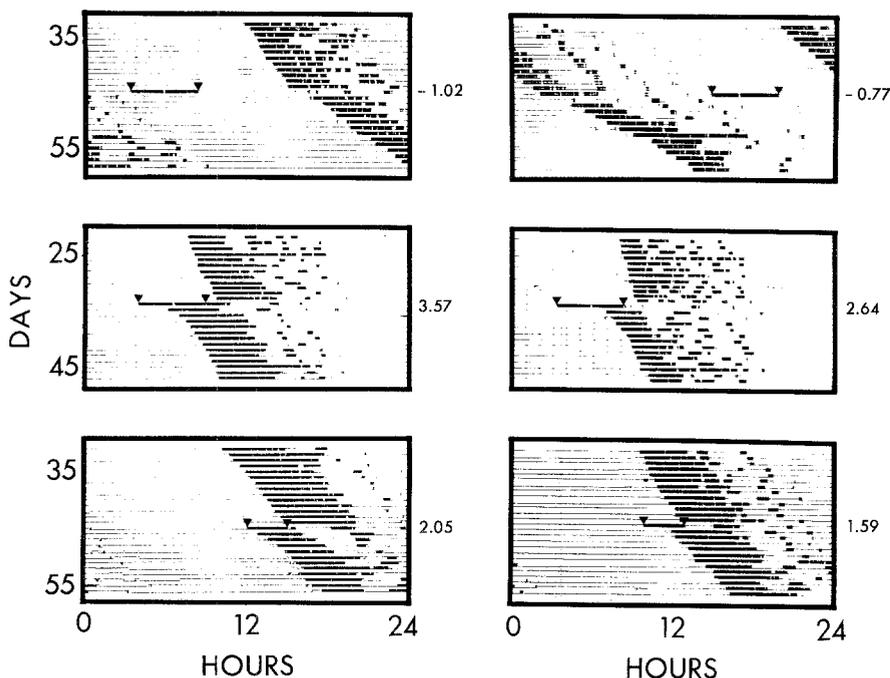
| Pulse       | Phase-shift (h)               |                              | $\tau$ change (h)           | n  |
|-------------|-------------------------------|------------------------------|-----------------------------|----|
|             | Immediate                     | Steady-state                 |                             |    |
| 5 h at CT 0 | $-1.1 \pm 0.4$<br>(-1.6--0.3) | $-0.6 \pm 0.6$<br>(-1.4-0.7) | $0.3 \pm 0.1$<br>(0.2-0.7)  | 10 |
| 5 h at CT 6 | $3.6 \pm 1.5$<br>(1.2-6.4)    | $3.5 \pm 1.2$<br>(1.4-5.6)   | $0.2 \pm 0.2$<br>(-0.1-0.4) | 13 |
| 3 h at CT 9 | $2.2 \pm 0.9$<br>(0.9-3.6)    | $2.3 \pm 1.0$<br>(1.2-4.1)   | $0.1 \pm 0.1$<br>(-0.2-0.2) | 7  |

maximum intensity was 166 lux and minimum was 78 lux. We therefore assert that the phase-shifts obtained here were caused mostly by exercise, and consequently that induced activity can cause phase-shifts in LL. This conclusion is consistent with the idea that running activity can contribute to the phase-shifting effects of dark pulses, given that animals often run during dark pulses.

How do our phase-shifts after induced wheel-running compare with phase-shifts after dark pulses? Boulos and Rusak (1982) and Ellis et al. (1982) have studied the effects of dark pulses administered to Syrian hamsters in LL. Their methodology differed somewhat from ours (e.g. individual hamsters receiving more than 2 pulses over a period of up to 1 year, slight differences in methods of phase-shift calculation) but the light intensities

they used were similar to ours. With these caveats in mind, one can compare the amplitude of their phase-shifts in response to dark pulses with our activity-induced shifts. 6-h dark pulses given around CT 6 resulted in steady-state phase-advances ranging between 0.9 and 5.5 h, and averaging about 3.6 h (from Fig. 4 in Boulos and Rusak 1982). These amplitudes do not appear different from those obtained here with 5-h activity pulses at CT 6 (see Table 1). 3-h dark pulses given around CT 9 yielded phase-advances ranging between 0 and 2.2 h and averaging about 0.8 h (from Fig. 4 in Ellis et al. 1982; see also Boulos and Rusak 1982 for comparable results with 2-h pulses). These values are much less than those found here with 3-h activity pulses given at CT 9 (mean  $> 2$  h, Table 1). In general, these comparisons show that our activity-inducing manipulation can lead to phase-advances that are at least as large as those caused by dark pulses of similar duration. This suggests that a major portion, if not all, of the phase-advancing effects of dark pulses could be attributed to running activity.

Boulos and Rusak (1982) also found that 6-h dark pulses given near CT 0 resulted in steady-state phase-delays ranging between 1 and 4 h, and averaging about 2.8 h. This is clearly more than the phase-delays (0.6 h) obtained here with 5-h activity pulses starting at the same CT. Therefore running activity would appear to be capable of accounting for only a small part of the phase-delaying effects of dark pulses at CT 0. However, phase-delay re-



**Fig. 1.** Actograms from hamsters free-running in LL and given pulses of induced wheel-running (between tips of triangles). Numbers on the right show the calculated phase-shifts in h (negative numbers indicate delays, positive numbers indicate advances). Top row: 5-h pulses starting at CT 0; middle row; 5-h pulses starting at CT 6; bottom row: 3-h pulses starting at CT 9

sponses are often inconsistent. For example, neither Ellis et al. (1982) nor Albers (1986) obtained clear phase-delay sections in their PRC to darkness, and Mrosovsky (1988) obtained phase-delay responses of different amplitude in response to various non-photoc manipulations. More research is necessary to define the conditions in which phase-delay responses can be reliably obtained. From here on, this paper will concentrate on phase-advancing effects.

## II. Restricting activity during dark pulses

If running activity is involved in the phase-advancing effects of dark-pulses, then animals that do not run during a dark pulse, whether by choice or because they are prevented from running, should phase-shift less, if at all. In a preliminary experiment we obtained evidence that hamsters that voluntarily refrain from running during a dark pulse indeed do not phase-shift. A 3-h dark pulse was given to 6 hamsters that were 195 days old and had been in LL for 10 days (during the 135 days that preceded LL, these hamsters had been exposed to an LD 14:10 cycle that had been phase-advanced twice by 8 h). At a time corresponding to CT 5–7, the lights were switched off from outside the room, and turned back on again 3 h later. For unknown reasons (possibly age or previous photoperiodic history) the hamsters were not aroused by this dark pulse, barely ran if at all, and did not appear to shift (the mean immediate shift was in fact a delay of 0.4 h). This observation encouraged us to look at the effects of imposed activity restriction during dark pulses and its influence on phase-shift amplitude.

### Material and methods

Two experiments were performed. Both were conducted in the same room (temperature ca. 21 °C). Both involved 60-day-old male hamsters (HsD:SYR from Sprague-Dawley Michigan in the first experiment, and LAK:LVG from Charles River Quebec in the second). In both cases we left the hamsters in LL for 15–19 days, and then gave them a 3-h pulse of darkness starting at CT 9 by lowering a wooden box, with lightproof louvers for ventilation, over individual cages.

The two experiments differed in the type of cage used, in the light intensity inside those cages, and in the way that activity was restricted during the dark pulses. In the first experiment, the same metal-walled cages as before were used. Light intensity varied from 30–75 lux at wheel level to 15–25 lux in corners. For half of the animals the wheel was locked from outside the cage during the dark pulses. These hamsters could still climb into the wheel, but could not move it. The unrestricted animals could run freely in their wheel.

In the second experiment, cages made of plexiglass were used (Fig. 2). These cages comprised 3 sections: nest box, open

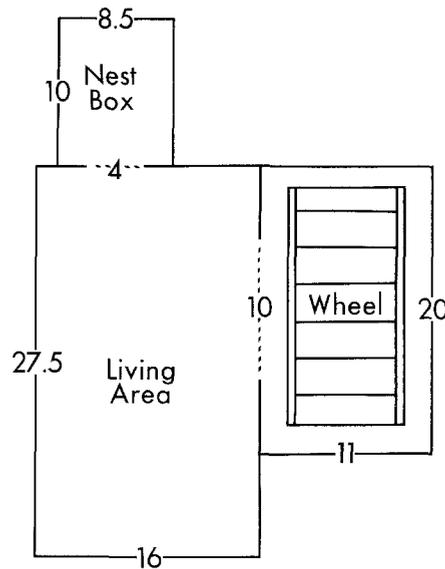


Fig. 2. Floor plan of the plexiglass cage used in some of the activity restriction experiments. Numbers refer to dimensions in cm

area, and running wheel. Light intensity inside these sections was 80–100 lux. All hamsters slept and stored food in the nest box. Just before the dark pulse, we confined half of the animals to their nest box by closing a sliding door at its exit. This operation awoke the hamsters, and therefore they saw the light-to-dark transition that followed. The unrestricted hamsters could leave the nest box at any time and had access to their wheel, but not to water (their water bottle was removed to control for the lack of water access in the animals confined to their nest box).

Phase-shifts were calculated as above. For 7 animals (4 in the first experiment, 3 in the second), no phase-shift could be calculated because the rhythms split within 7 days post-pulse. Statistical tests followed Conover (1980) and were one-tailed only when testing the prediction that activity-restricted hamsters should phase-shift less than unrestricted hamsters.

### Results

In the first experiment (metal-walled cages), preventing the wheel from rotating during the dark pulses reduced the steady-state phase-shifts by about 50% (Table 2A, Fig. 3 top row). All animals in the unrestrained group ran for more than half of the time they were in the dark (see Fig. 3).  $\tau$  always increased after a dark pulse, but the increase was similar in both restrained and unrestrained groups (Table 2A).

In the second experiment (plexiglass cages), confining the hamsters to nest boxes during the dark pulses abolished the phase-shifts (Table 2B, Fig. 3 bottom row). All animals in the unrestrained group ran for more than 75% of the time spent in the dark (see Fig. 3).  $\tau$  always increased after a dark pulse, but the increase was similar in both restrained and unrestrained groups (Table 2B).

**Table 2.** Effects of 3-h dark pulses (starting at CT 9) on the activity rhythms of hamsters that were either free to run in their wheels during the pulse or prevented from doing so. Shown are means  $\pm$  SD. The result of a statistical test for difference between unrestrained and restrained group is shown on the right. NS non-significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.005$

|                        | Group         |                | Mann-Whitney <i>T</i> |
|------------------------|---------------|----------------|-----------------------|
|                        | Unrestrained  | Restrained     |                       |
| A) In metal cages      |               |                |                       |
| Immediate shift (h)    | 1.3 $\pm$ 0.5 | 0.9 $\pm$ 0.2  | 58 NS                 |
| Steady-state shift (h) | 1.1 $\pm$ 0.5 | 0.6 $\pm$ 0.3  | 51.5*                 |
| $\tau$ change (h)      | 0.2 $\pm$ 0.1 | 0.2 $\pm$ 0.1  | 69 NS                 |
| <i>n</i>               | 8             | 9              |                       |
| B) In plexiglass cages |               |                |                       |
| Immediate shift (h)    | 1.4 $\pm$ 0.7 | -0.4 $\pm$ 0.2 | 28***                 |
| Steady-state shift (h) | 1.1 $\pm$ 0.8 | -0.1 $\pm$ 0.4 | 32**                  |
| $\tau$ change (h)      | 0.3 $\pm$ 0.1 | 0.2 $\pm$ 0.1  | 38 NS                 |
| <i>n</i>               | 7             | 7              |                       |

## Discussion

The restriction of activity during dark pulses decreased shift amplitude. This decrease was less pronounced in the experiment with metal cages. This is not unexpected because the level of restriction was not maximum: although the hamsters could not run in the wheel, they could still walk about in their cage, climb onto their wheel, etc. In the experiment with plexiglass cages, the hamsters were confined to their sleeping quarters and were presumably completely inactive (we heard no sounds of gnawing or scratching, which in unrestrained animals could be heard through the ventilation system of the boxes). The phase-advances

were then completely abolished. This suggests that 3-h dark pulses at CT 9 phase-shift rhythms not because of a change in photic input to the internal clock but rather because of the activity they trigger.

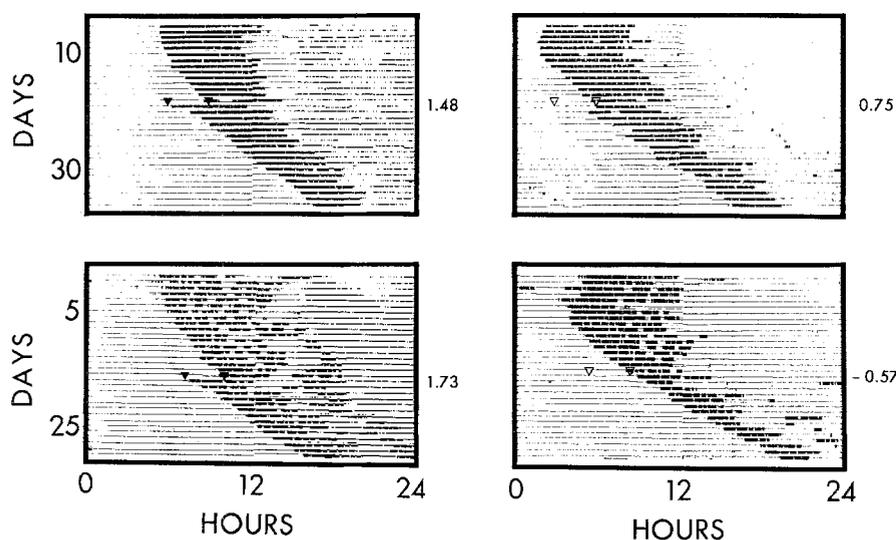
There is, however, an alternative interpretation. Perhaps activity restriction in itself has phase-delaying effects, and these may have simply cancelled out the photic, phase-advancing, effects of the dark pulses. It is conceivable that the main feature of a dark pulse is the light-to-dark transition at its onset, and that this photic feature immediately phase-advanced the rhythms by 1.1 h in our two experiments. The activity restriction would then have coincided approximately with the last 2 h of the subjective day and the first 1 h of the subjective night. It is not known whether activity restriction at these times has any phase-shifting effect. We investigated this question in the following experiment.

## III. Restricting activity without dark pulses

### Material and methods

Male hamsters (HsD:SYR, from Sprague-Dawley Michigan) were put into LL when 62 days old. Some ( $n=12$ ) of these hamsters were in metal-walled cages, while others ( $n=8$ ) were in plexiglass cages. Light intensity was 90–150 lux inside the metal-walled cages, and 40–60 lux in the plexiglass cages.

On day 17–22 after LL initiation, each hamster was given a single 3-h pulse of activity restriction starting 2 h before CT 12. These pulses coincided with the last 2 h of the subjective day and the first 1 h of the subjective night. As before, animals in the metal cages were restrained by having their wheels locked from the outside of the cage, while animals in plexiglass cages were confined to their nest boxes. These manipulations entailed no change in light intensity. Phase-shifts were calculated as above. For 2 animals from the metal cages and 3 animals from



**Fig. 3.** Actograms from hamsters free-running in LL and given 3-h dark pulses (between tips of triangles) starting at CT 9. Some of the hamsters could run during the pulses (closed triangles) while others were prevented from running (open triangles). Top row: hamsters kept in metal cages; bottom row: hamsters kept in plexiglass cages. Numbers on the right show the calculated phase-shifts in h

the plexiglass cages, no phase-shift could be calculated because the rhythms split within 7 days post-pulse.

## Results

In both metal and plexiglass cages, activity restriction resulted in immediate phase-delays of about 30 min on average, in steady-state phase-delays of only 5–10 min on average, and in  $\tau$  increases of only 8–9 min on average (Table 3, Fig. 4). The results with both cage types do not differ significantly from each other ( $P > 0.05$ , Table 3). Direct observation of the animals' behaviour revealed that they were more or less inactive during the first half of the pulse, but active in the second half, i.e. when normally they would have been wheel-running. In the metal cages, this activity consisted of grooming, walking in and out of the locked wheel, and climbing to the top of the wheel. In the plexiglass cages, hamsters groomed or gnawed

and scratched at the door and walls of the nest box.

## Discussion

The average steady-state phase-delays caused by the activity restriction amounted to only a few minutes. This is too marginal an effect to lend support to the idea that, in our previous experiments, activity restriction had phase-delaying effects cancelling out a photically-induced phase-advance of 1.1 h. Moreover, whereas animals in this experiment attempted to remain active despite the restriction, at least at a time when their clock dictated it (second half of the pulse), restrained hamsters in the previous experiments gave no signs of being active at any time of the manipulation. This suggests that their clock was at a phase that did not dictate activity, and therefore that it had not been phase-advanced.

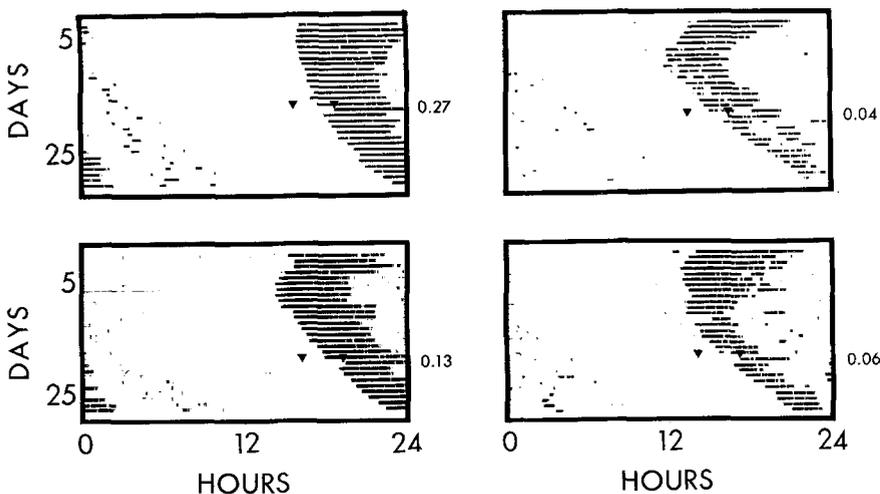
Activity restriction, however, did have some short-term effects, as indicated by non-negligible immediate delays (Table 3). It would be interesting to test activity restriction at other circadian times and with longer pulse durations, in the hope of obtaining more permanent effects on rhythms. Bouts of induced wheel-running produce large phase-shifts only when administered for several hours during the subjective day (Reebbs and Mrosovsky 1989b), and maybe long durations are also necessary for activity restriction to cause phase-shifts during the subjective night.

**Table 3.** Effects of 3-h pulses of activity restriction (starting 2 h before CT 12) on the activity rhythms of hamsters in LL. Shown are means  $\pm$  SD, with range in parentheses. One group (metal cages) had their wheels locked as a means of activity restriction, whereas the other group (plexiglass cages) were confined to nest boxes. The result of a statistical test for difference between the two groups is shown on the right; *NS*, non-significant

|                        | Group                         |                               | Mann-Whitney <i>T</i> |
|------------------------|-------------------------------|-------------------------------|-----------------------|
|                        | Metal cages                   | Plexiglass cages              |                       |
| Immediate shift (h)    | $-0.5 \pm 0.3$<br>(-0.9-0.0)  | $-0.5 \pm 0.2$<br>(-0.7--0.3) | 37 NS                 |
| Steady-state shift (h) | $-0.1 \pm 0.3$<br>(-0.5--0.3) | $-0.15 \pm 0.2$<br>(-0.4-0.1) | 45 NS                 |
| $\tau$ change (h)      | $0.2 \pm 0.1$<br>(-0.1-0.4)   | $0.1 \pm 0.1$<br>(0.1-0.2)    | 33 NS                 |
| <i>n</i>               | 10                            | 5                             |                       |

## General discussion

Confining hamsters to nest boxes blocked the phase-shifts caused by 3-h dark pulses at CT 9. The same manipulation without dark pulses had no major effect. This, taken together with the find-



**Fig. 4.** Actograms from hamsters free-running in LL and given 3-h pulses of activity restriction (between tip of triangles) starting 2-h before CT 12. The method for activity restriction was wheel-locking (metal cages, top row) or confinement in a nest box (plexiglass cages, bottom row). Numbers on the right show the calculated phase-shifts in h

ing that 3-h dark pulses of induced running alone produce phase-shifts at least as large as those caused by 3-h dark pulses at CT 9, is strong evidence that dark pulses phase-advance rhythms because of their influence on behavioural states rather than on photic input.

How well this conclusion applies to other phases of the circadian cycle, other pulse durations, other background illuminations, and other species is an open question available for future investigation. At least for CT 6, the effect of dark pulses in hamsters is probably also mediated by activity; our 5-h pulses of induced wheel-running at CT 6 produced phase-advances as large as those caused by 6-h dark pulses given near the same CT. We tried several times to apply an activity restriction paradigm to animals given 6-h dark pulses at CT 6, but those experiments were plagued by anomalous responses such as splitting, appearance of secondary components, wavy baseline of activity onsets, and lack of shifting in unrestrained animals. However, concurrent work by van Reeth and Turek (1989) has shown that complete immobilization blocks the phase-shifting effects of 6-h dark pulses given at CT 6 on a background illumination of 600 lux.

The behavioural mediation reported here points to the importance of observing and measuring behaviour during manipulations that alter rhythms, and of considering behaviour as a causal factor rather than as a mere concurrent variable. One should not assume that the change in behaviour has to be an increase in activity. For example, in diurnal animals given dark pulses the behavioural change could be a decrease in activity (a natural response to darkness by light-adapted animals). For example, Wahlström (1965) imposed periods of prolonged darkness early in the subjective day of canaries, *Serinus canaria*, that were self-selecting their own LD cycle. Immediate delays of up to 5 h were observed, but it was evident that the dark period affected the behaviour of the birds, increasing their rest time. Similarly, Klein et al. (1985) found that dark pulses led to phase-advances when given in the early subjective night to house sparrows, *Passer domesticus*, that had just been transferred into LL. Here again, pulses of darkness prevented the birds from being active (see Fig. 1H in Klein et al. 1985); to complicate things, some sparrows also showed extra activity when transferred into LL (see Fig. 1G in Klein et al. 1985), and the possibility arises that the phase-shifts were causally related to this extra activity.

In studies devoted to non-behavioural effects, behaviour alteration would have to be somehow prevented during manipulations. This could be

done by direct intervention, or by choosing species whose behaviour is not altered by the manipulations. In the case of dark pulses and diurnal animals, the eastern chipmunk, *Tamias striatus*, may represent such a species. Preliminary work by DeCoursey (1973) has indicated that 1-h dark pulses given early in the subjective day of a chipmunk delayed its activity rhythm, even though the chipmunk kept on wheel-running (as it normally did at that circadian time) throughout the pulse. Similar observations were made by Navaneethakannan and Chandrashekar (1986) on the palm squirrel, *Funambulus palmarum*, except that dark pulses in the early subjective day led to phase-advances.

That the phase-shifting effects of dark pulses may be behaviourally mediated also has theoretical implications. Periods of darkness involve light-to-dark and/or dark-to-light transitions. These transitions are obvious photic events that could be detected by the internal pacemaker and used as phasic signals to help synchronize the animal to an LD cycle (Moore-Ede et al. 1982, p. 92–93). One might expect transitions in opposite directions to give opposite phase-shifts. This expectation has generated attempts to show that PRCs for LL-to-DD and DD-to-LL are mirror images of each other (the same has been done for light and dark pulses, but the pulses were not of the same duration, and therefore comparisons were not strictly valid). One study (Subbaraj and Chandrashekar 1981) found an almost perfect mirror image, but another (Albers 1986) found it to be limited to the subjective day. This discrepancy undermines the expectation of opposite transitions giving opposite results. One can go one step further and question whether light-to-dark and dark-to-light transitions are used at all by the pacemaker as synchronizing signals. The absence of phase-shifts in activity-restricted hamsters after dark pulses (two single-step transitions) suggests that indeed single-step transitions may not be used by the pacemaker. This does not mean, however, that gradual transitions could not affect the circadian system. Kavanau (1962) has shown that the presence of dawn- and dusk-like transitions instead of single-step transitions helped deer mice, *Peromyscus maniculatus*, entrain better to an LD cycle of extreme periodicity (16 h). In future research, it may be useful to study the phase-shifting effects of dark pulses with gradual onsets, in both restrained and unrestrained animals.

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