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Aftereffects of Scheduled Daily Exercise on Free-Running Circadian Period in Syrian Hamsters

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REEBS, S. G. AND J. ST-COEUR. *Aftereffects of scheduled daily exercise on free-running circadian period in Syrian hamsters.* *PHYSIOL BEHAV* 55(6) 1113-1117, 1994.—This study examined whether a nonphotic factor, scheduled daily exercise, could cause aftereffects on the free-running circadian period of Syrian hamsters. Groups of hamsters were kept under a cycle of 14-h light:10-h dark with access to their running wheel for only 3 h a day. Depending on the group, this 3-h period coincided with early day, midday, late day, early night, or late night. Controls did not have access to wheels. After 12 days, all hamsters were released into constant darkness (DD) and given free access to their wheel. Late-day runners showed a significantly shorter free-running period in DD compared to night runners and to controls, indicating that free-running periods can be shortened by nonphotic factors in this species. On the first day of DD, the activity onset of hamsters preceded (midday and late-day runners), coincided with (night runners and controls), or followed (early day runners) the previous time of D onset. Advanced activity onsets in late-day runners were consistent with both their short free-running periods and probable phase-advancing effects of late-day exercise; in contrast, delayed activity onsets in early day runners could only be explained by phase-delaying effects of the scheduled exercise.

Exercise Aftereffect Free-running period Phase relationship Wheel running Circadian rhythms
 Syrian hamster

IN recent years, increasing attention has been given to the different ways in which behavior-altering manipulations can affect circadian clocks in animals (15,22). One behavior-altering manipulation that is easy to perform on captive rodents is to give access to a new running wheel for a limited time. This procedure normally induces spontaneous running. Administered daily in otherwise constant conditions, wheel access can entrain activity rhythms in hamsters (19) and mice (5). In hamsters, single bouts of wheel access can also phase-shift rhythms (16,19,23) and cause changes in the free-running period, τ (14). Daily wheel running at specific times can also cause aftereffects on τ . For example, Mistlberger (11) repeatedly confined hamsters in a running wheel during the last hour of the scotophase and the first 2 h of the photophase under a cycle of 14-h light:10-h dark (LD 14:10), and relative to unconfined controls he observed longer τ s when the hamsters were subsequently released in constant darkness (DD). In contrast, confinement in the middle of the day did not affect τ .

The lack of aftereffects following midday confinements to a running wheel seems surprising because subjective day is a time when single bouts of induced running normally cause phase-shifts (16,19,23) and τ changes (14). A possible explanation for the lack of effect is that the hamsters in Mistlberger's (11) study did not run sufficiently in their wheels. It is known that a minimum threshold of running is necessary for nonphotic effects to take place (10,19). In the present study, we repeated and expanded Mistlberger's study with some protocol alterations meant

to increase running activity during wheel confinement. We also increased the number of circadian times tested. Our aim was to identify times at which daily scheduled exercise in LD could significantly affect τ in subsequent DD relative to unexercised controls.

One interest of studying aftereffects on τ is that, based on the theory of oscillator entrainment (1), one should expect changes in the phase relationship between activity and LD cycle when τ changes. In theory, shorter τ s should result in activity onsets that are more advanced, or less delayed, relative to light or dark onset. An interesting implication, from a human perspective, is that daily manipulations that can shorten τ might help transform late risers into early risers. Note, however, that advanced activity onsets could also result from daily phase-shifts caused by the recurrent manipulation (11), without any necessary modification of τ . Our study was not originally designed to tease apart phase-shift and τ effects as determinants of phase relationship, but we nevertheless estimated the timing of activity onset relative to dark onset, and considered whether this phase relationship was consistent with phase-shift effects, τ effects, or both.

METHOD

Animals and Housing Conditions

Male Syrian hamsters (*Mesocricetus auratus*, LVG; from Charles River Canada, Montreal; 60 days old) were housed singly in Wahman cages (25 × 15 × 13 cm) connected to running

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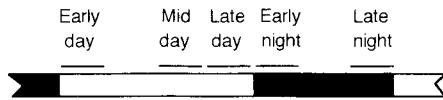


FIG. 1. Daily times at which different groups of hamsters were confined to running wheels, for 12 consecutive days before being released into DD. Each horizontal line represent a 3-h confinement associated with a specific group.

wheels (36 cm in diameter). Water, rodent lab chow, and 5.6 g of cotton bedding material were available in each cage. Room temperature was $21 \pm 1^\circ\text{C}$, and lighting came from overhead incandescent bulbs. Light intensity, measured with a Gossen Lunasix 3 light meter, approximated 50 lux inside the cages during L, and 0 lux during D.

A sliding door between cage and wheel allowed us to control wheel access. All running wheels were equipped with microswitches connected to a computer. Wheel revolutions were recorded in 6-min bins using Dataquest III hardware and software (Data Sciences Inc., available from Mini-Mitter Co., Sunriver, OR).

Protocol

During the first 17 days of the experiment the hamsters were kept under LD 14:10. During the first 5 of these days they had no access to their running wheel (adaptation period). During the next 12 days they had access to their wheel for only 3 h a day (this limited access was our means of increasing running activity). During those 3 h the hamsters were confined in the wheel, had no access to food or water, and were not forced to run. Five groups were formed ($n = 12\text{--}13$ per group). In these groups, daily confinement inside the wheel began, respectively, at day onset, 6 h before night onset, 3 h before night onset, at night onset, and 3 h before day onset. These groups were called early day, midday, late day, early night, and late night, respectively (Fig. 1). The number of wheel revolutions during confinement was noted every day.

At the start of the first complete scotophase that followed the last of the 12 confinements, the hamsters were left in DD and given free access to their wheel. They were allowed to free-run in DD for a minimum of 10 days.

A sixth group of hamsters ($n = 14$) was treated in the same way as the others except that these hamsters never had access to their wheel before DD. This was a control group for the lack of normal running activity at night.

Data Analysis

Dataquest data were downloaded to another computer for plotting and analysis with the TAU program (J. Schull, available from Mini-Mitter Co., Sunriver, OR). For each hamster, the first three daily activity onsets in DD were ignored but the times of the following seven were used to calculate a least-square regression line, the slope of which was added to 24 to give τ . We also fitted a regression line to the second–fifth activity onsets in DD and extrapolated this line to the day of DD onset; the difference between the extrapolation and the time of DD onset (which was the same time as D onset under LD) was taken as an estimate of the phase relationship between activity rhythm and LD cycle at the end of the 12 days of treatment.

Statistical analyses involved Kruskal–Wallis tests followed by nonparametric multiple comparisons (3). We used nonparametric procedures because of persistent heterogeneity of variances in the phase relationship data set. We tried ANOVAs fol-

lowed by least-square difference comparisons (SPSS-PC) on the wheel revolution and τ data sets (where variances were homogeneous), and obtained the same results as in the nonparametric analyses; for brevity, we present only the nonparametric statistics.

RESULTS

On average and in all experimental groups, hamsters ran more than 1000 wheel revolutions per hour during wheel confinement [Fig. 2(A)]. However, some groups ran more than others (Kruskal–Wallis' $t = 30.1$, $p < 0.0001$). The least active runners were in the midday group, followed by the late-day group, followed by the other three groups [Fig. 2(A)].

Groups also differed with respect to τ after release in DD ($t = 18.4$, $p < 0.003$). Midday and late-day runners had τ s that were significantly shorter than night runners [Figs. 2(B), 3]. However, of all the groups, only the late-day runners had τ s significantly different (shorter) than those of the control group [Fig. 2(B)].

Phase relationship between activity rhythms and LD cycle also varied according to groups ($t = 40.5$, $p < 0.0001$). Activity

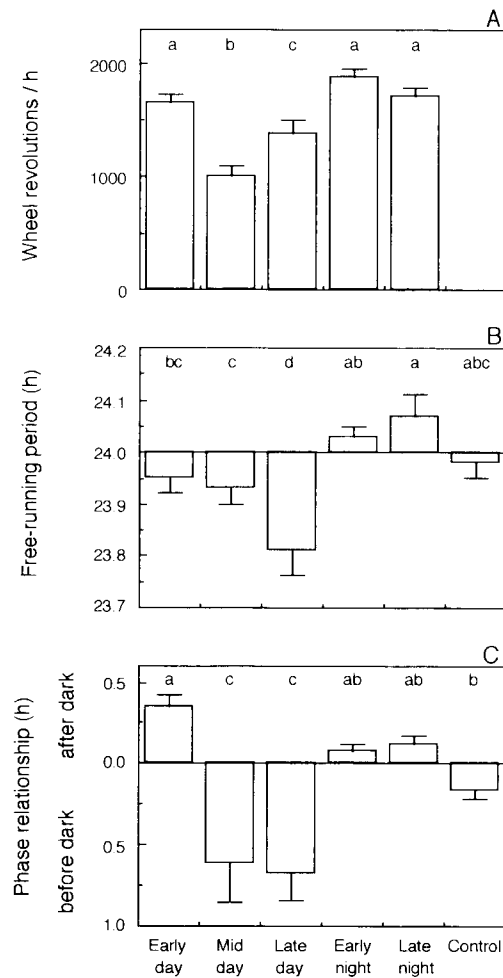


FIG. 2. Mean \pm SE ($n = 12\text{--}14$) running activity during confinement (A), subsequent free-running period in DD (B), and time of activity onset relative to D onset on the last day in LD (C), as a function of the time of daily wheel confinement in LD. Within each panel, groups that do not share a letter are significantly different.

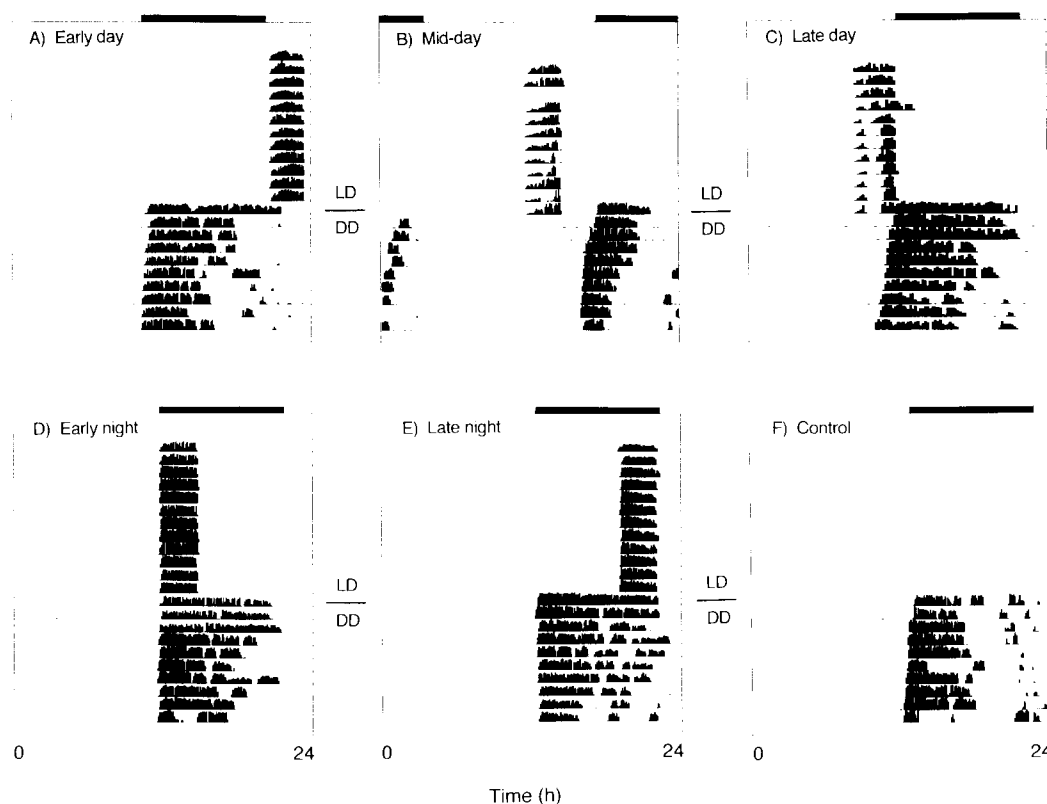


FIG. 3. Actograms from the early day (A), midday (B), late-day (C), early night (D), late-night (E) and control groups (F). Dark areas above each actogram represent night in the LD cycle that preceded DD. Actograms were generated by the TAU program, setting C (ceiling, or the y-axis value of the highest possible bar) at 300 wheel revolutions per 6 min.

preceded dark onset in the midday and late-day runners, almost coincided with dark onset in controls and night runners, and followed dark onset in early day runners [Figs. 2(C), 3]. Within each group, we could not find a significant positive correlation between phase relationship and τ , but when data from all six groups were pooled, we did (Spearman's $\rho = 0.2407$, $n = 75$, $p < 0.02$).

DISCUSSION

Amount of Running

Was our protocol successful in eliciting sufficient running from all groups? The answer seems to be yes. Janik and Mrosovsky (10) have shown that consistently large phase-shifts are obtained when hamsters run at least 1666 revolutions per hour during 3-h wheel confinements [see also (13) for a lower threshold of 1333 revolutions per hour]. Considering that these authors used wheels that were 17.5 cm in diameter and we used wheels of 36 cm diameter, their threshold translates to at most 810 revolutions per hour for our study. This value is consistent with unpublished experiments from our laboratory in which we found a threshold of 830 revolutions per hour for consistent phase-shifts. All of our noncontrol groups ran at least 23% more than this on average. It is clear, however, that hamsters ran relatively less during midday and late-day confinement (but, interestingly, not during early day confinement). This could be explained by a greater sleep tendency in hamsters during the second half of the day as opposed to nighttime or early day (21). Note, however, that a recent study (8) has indicated a sleep tendency that was no

lower in early day than in late day, although both were lower than at midday (unfortunately in this case, manipulations at the onset of the 24-h recording may have advanced the activity rhythm, bringing the low sleep tendency of early night into the late-day part of the LD cycle).

Effect on τ

Hamsters that ran during the night, their normal activity time, showed subsequent τ values (24.03 h and 24.07 h) similar to those measured in other studies where hamsters had free access to their wheels at all times [e.g., 24.04 h in (4), 24.05 h in (11), 24.11 h in (7), 24.12 h in previous experiments in our laboratory]. In contrast, midday and late-day runners showed significantly shorter τ s (23.93 h and 23.81 h, respectively), and early day runners also had shorter τ s (23.95 h), albeit not significantly so. This seems to indicate that day running has a shortening aftereffect on τ . However, this statement must be qualified: it may not truly apply to early day and midday running, because these two groups did not differ significantly from the nonrunning controls (23.98 h). Therefore, short τ s in these groups may have resulted from the absence of night running rather than the presence of day running. There is independent evidence that lack of wheel access at night can shorten τ (2). Thus, only in the case of the late-day runners can we be confident that day running had a shortening aftereffect on τ . This result is novel and indicates that τ can be not only lengthened (11) but also shortened by appropriately scheduled exercise in LD.

Interestingly, single pulses of wheel access in constant conditions do not usually shorten τ (14). Shortening of τ may thus

be limited to our present conditions of consecutive daily manipulations in LD. The minimum number of consecutive days necessary to obtain the effect is unknown but is currently under investigation in our laboratory. The role of LD is also uncertain but it may not be overly important. In a previous study (19), all 10 hamsters that entrained, in DD, to wheel confinement in late subjective day had shorter τ s after entrainment than before.

Also interesting is the fact that none of our groups showed significant τ lengthening relative to controls. In particular, the relatively short τ s exhibited by the early day group were unexpected. In his experiments, Mistlberger (11) had a group for which running was scheduled only 1 h before that of our early day group, and he observed τ s (24.24 h on average) that were significantly longer than in controls. Part of the difference between his early day runners and ours may have stemmed from the forced absence of nocturnal running in our early day group, resulting in shorter τ s. Aftereffects on τ caused by nocturnal inactivity are suggested by the (nonsignificantly) shorter τ s observed in our controls relative to night runners, and by a previous study that has shown significantly shorter τ s associated with the lack of wheel availability in hamsters [(2); see also (6) and (20) for inverse relationships in mice and rats, respectively]. However, this argument does not explain why our early day runners did not show long τ s relative to our own controls. Possibly, early day is an unstable time for nonphotic responses in hamsters [it corresponds to the passage from slight phase-delays to large phase-advances on the phase-response curve to induced running (19)] and the 1-h difference in scheduling may therefore be important.

Effect on Phase Relationship

We measured phase relationship between activity onset and dark onset by extrapolating the free-running rhythm in DD back to the last day of LD. Phase relationship could not be measured directly under LD because wheel access was strictly controlled at that time. Our method, however, has the advantage of being unaffected by the masking effects that light often has on activity (an important consideration when one expects activity onset to precede dark onset). Nevertheless, in the following discussion, one should bear in mind that our measure is only an estimate of the phase relationship on the last day of LD, and not necessarily the exact relationship that might have been observed on every single day of a continuing LD cycle.

As in Mistlberger's earlier study (11), we found that, on the first day of DD, the activity rhythm of early day runners was delayed relative to the previous LD cycle. As opposed to that study (11), however, but in accordance with a later report (12), our midday runners showed a significantly advanced activity onset relative to the previous LD cycle. The difference with Mistlberger's earlier study (11) is probably related to an increased motivation to run by our hamsters due to the lack of wheel availability at other times. [Motivation to run, as opposed to forced running, seems to be an important component of nonphotic effects (10,11).] Similarly, our late-day runners also showed advanced activity relative to night onset. As could be expected from the theory of oscillator entrainment, our two groups with the shortest average τ s also showed the most advanced activity onset relative to dark onset, and there was a positive correlation between τ and phase relationship when data from all groups were pooled. It is difficult, however, to determine whether phase relationship in midday and late-day runners resulted from the short τ s only or also from daily phase-shifting of activity caused by the pulses of running. We already know that induced activity in mid or late subjective day can phase-advance free-running rhythms (16,19,23). The observed phase relation-

ship is therefore consistent with both phase-shift effects and τ effects.

We also know that wheel confinement early in the subjective day can phase-delay activity rhythms (19). Consistent with this, our early day runners showed the most delayed activity, and they did so without exhibiting long τ s (most likely because of the lack of nocturnal running). Therefore, in this case it appears that phase relationship was affected only by daily phase-shifting.

Implications and Future Studies

Together with previous experiments, our results show that daily scheduled exercise can have both shortening and lengthening aftereffects on τ ; our results also suggest that phase relationship during entrainment to LD could be affected by scheduled exercise. It would be worthwhile, in future experiments, to determine whether changes in phase relationship are caused mostly by changes in τ or by daily phase-shifts. For example, humans who run in the morning show an altered phase relationship between their body temperature cycle and the day-night cycle (17). Is this effect caused by daily phase-shifting or by changes in τ ? An important point is that aftereffects on τ tend to be long term [sometimes months in mice (18)], whereas phase-shift effects are short term (the day of the phase-shift at least, or the duration of transient cycles at most). Long-term effects are bound to be more attractive to humans because then the (possibly annoying) scheduled exercise would need only be done for a few days to enjoy, for a hopefully long time afterwards, the desired phase relationship with the day-night cycle.

It remains to be seen, however, how persistent the aftereffect on τ could be, especially if 24-h LD cycles were to be maintained after the scheduled exercise, as would be the case in humans. The problem here is that LD cycles themselves can have aftereffects on τ (18) and thus may cause τ to come back to its original preexercise level. Experimentally, this could be addressed easily enough by keeping subjects under 24-h LD cycles of various durations after the series of exercise days, before release into DD.

A final question is whether τ -induced advanced activity could be negated by phase-delaying effects of light during the LD cycle that would follow exercise days. This question is particularly relevant in the case of hamsters, because for these nocturnal animals advanced activity onsets may mean being exposed to the light of late day, a time when light phase-delays rhythms (4). In humans, the situation is not so clear: rising earlier would not expose them to more light in the morning [and even if it did, this would only help them to rise early, because early morning light phase-advances rhythms; see (9)]. On the other hand, one might argue that light in the late day would now fall on a later part of their phase-response curve, one that is more susceptible to cause phase-delays; it seems, however, that light pulses have little effect on human circadian rhythms in late subjective day or early subjective night (9), although small sample sizes preclude any firm conclusion. Incertitude here points to the need to carry out more work, both in rodents and humans, to find effective ways to maintain desired phase relationships between activity and LD cycles.

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