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Phase Angle Changes of Photically Entrained Circadian Rhythms Following a Single Nonphotic Stimulus

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JANIK, D., M. GODFREY AND N. MROSOVSKY. *Phase angle changes of photically entrained circadian rhythms following a single nonphotic stimulus.* PHYSIOL BEHAV 55(1) 103–107, 1994.—Syrian hamsters entrained to a light–dark (LD) cycle of 14:10 h were given the opportunity to run in novel wheels for 3 h in the middle of the light phase. This manipulation transiently altered the phase angle of entrainment to the LD cycle: activity onset was significantly advanced (by about 0.5 h) on the day after the pulse and gradually drifted back toward its prepulse time. When animals were held in LD 11.5:12.5 h, a photoperiod in which onset time occurs later relative to the time of lights-off, they again advanced about 0.5 h in response to the pulse of wheel running, but many animals retained an advanced phase angle for at least 7 days, and some for more than 21 days. Individual changes in phase angle were highly correlated with the prepulse phase angle: the more negative the phase angle, the greater the advance subsequent to the novel wheel pulse. These results show that a single, short-duration, nonphotic manipulation can produce long-lasting alterations in the phase angle of entrainment to a LD cycle.

Activity Nonphotic Circadian Entrainment Phase angle Hamster

THE daily cycle of light and darkness is the main environmental cue organisms use to synchronize circadian rhythms to the 24-h day. The light–dark (LD) cycle not only has a synchronizing function, but also a direct excitatory or inhibitory influence. Together these two effects determine, to a great extent, the time of day at which an animal is active (1).

In recent years it has been shown that nonphotic stimuli, such as confinement to a novel wheel or exposure to sexual cues, can shift the phase of circadian rhythms in constant conditions and accelerate reentrainment to a shift in the LD cycle (6,16,20.26). It also has been shown that nonphotic stimuli can entrain circadian rhythms (2,4,5,11,19,21). With the exception of bird song (5,11) and temperature (2), the specific types of stimulation used to elicit these effects are not normally periodic in nature. It might, therefore, be reasonable to conclude that they serve not as entraining agents (Zeitgebers), but rather as modulators of the phase angle of entrainment determined primarily by the LD cycle.

To understand the functional importance, if any, of nonphotic factors in the circadian biology of an organism, one must first determine the extent to which such stimuli can influence animals entrained to an LD cycle. In one such study, Honrado and Mrosovsky (6) showed that periodic exposure to sexually receptive and nonreceptive females transiently advanced activity onsets in male hamsters. The effects were small (an initial advance of about 20 min), but the stimulus conditions were probably not optimal. Mistlberger (12) has also shown that scheduled daily exercise or feeding alters phase angle of entrainment in hamsters, but the effects were limited to phase delays.

In the present study, we examined the potential of nonphotic stimulation to alter entrainment of circadian locomotor rhythms using 3 h of confinement in a novel running wheel in the middle of the day. This corresponds to the circadian phase where novelty-induced running results in maximum phase advances of about 3 h (19,20). Our results showed a significant effect of this manipulation on phase angle of entrainment (Ψ).

METHOD

Animals

Male Syrian hamsters (*Mesocricetus auratus*, Hsd:SYR) were purchased from Harlan Sprague–Dawley (Indianapolis, IN). On arrival in the laboratory at 61 days of age, animals were housed individually in metal-walled cages (36 × 20 × 30 cm) equipped with a running wheel (17.5 cm dia.). Food (5001 Rodent Chow) and water were continuously available except during pulses in the novel wheels. Room temperature ranged from 19 to 23°C over the course of the experiments. The average incident light

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intensity in the cage was about 10 lux during the light phase, as measured with a Gossen Lunasix 3 light meter.

Daily Activity Records

Wheel revolutions in the home cage were recorded by a microswitch connected to an Esterline–Angus event recorder. To generate activity records, each channel of the recorder paper was cut into 24-h strips and mounted one below the other on cardboard panels.

Activity Pulse Procedure

Each animal was transferred from its home cage to a novel running wheel from which it could not escape. The novel wheel was the same type found in the home cage, mounted in a Plexiglas frame. Pulse duration was 3 h. Ambient temperature during the pulse was 14°C. Because the pulse was conducted in the light (see below), we were concerned that the amount of running would be inhibited. Cool ambient temperature increases the amount of running in a novel wheel, but does not, by itself, shift the circadian activity rhythm (8). Moreover, phase shifts obtained with pulses at cool temperatures are similar to those at warm temperatures (8).

Novel wheels were loaded with hamsters and taken to an environmental chamber in an adjacent room. Light intensity in the chamber was matched to that of the home cage room. During transport (ca. 2 min) to and from the chamber, the novel wheels were covered with a black cloth to prevent exposing the animals to a light intensity higher than that in the home cage room. Light intensity measured under the cloth ranged from 5 to 10 lux. Revolutions in novel wheels were registered on electronic counters.

Protocol

Animals were initially kept in LD 14:10 for 23 days to allow for entrainment. They were then given a novel wheel pulse at 14°C, 8 h before their projected activity onset. Eleven days later the LD cycle was changed to 11.5:12.5 (by delaying the lights-on time by 2.5 h) and 26 days were allowed for entrainment to this new LD cycle. Animals were then given another wheel pulse at 14°C, starting 8 h before their projected activity onset. For each test, about half the animals were kept undisturbed in their home cages.

Data Analysis

Daily onsets were defined as the first 5-min period of pen deflections without gaps followed by another such 5-min period within the next 0.5 h. Activity time (α) was estimated by visually fitting lines at activity onset and end for 7 days immediately prior to the pulse and for postpulse days 4 through 10. Two animals were excluded from the analyses: one animal had a low number of wheel revolutions (2112) during the second activity pulse; one animal was given a nonphotic pulse at the wrong time (second pulse). It was decided before the experiment that only animals running >4000 revolutions during a pulse would be considered in the analyses, since this was the minimum amount of running needed to elicit a large phase advance (13).

Two-way ANOVA was used to evaluate onset times for pulsed and control groups on successive days. The *t*-test and Bonferroni-corrected *t*-test (for multiple comparisons) were used to evaluate group differences on particular days. The Mann–Whitney *U*-test was used when the standard deviations between groups were significantly different. The Student–Newman–Keuls multiple range test (paired comparisons) was used to assess changes in onset time on successive days within groups. A two-tailed significance level of $p = 0.05$ was used unless indicated otherwise.

RESULTS

In LD 14:10 there was a significant effect of the nonphotic pulse on Ψ [$F(1, 119) = 13.6$, $p < 0.005$; Fig. 1]. Activity onsets of pulsed animals ($n = 15$) advanced about 20 min on postpulse day 1 and were significantly earlier as compared to home cage controls ($n = 5$; $p < 0.01$, Mann–Whitney *U*-test). This difference was maintained for at least 8 days after the pulse ($p < 0.05$, *t*-test). Raw data illustrating this effect are shown in Fig. 2.

In LD 11.5:12.5 the nonphotic pulse ($n = 10$) significantly advanced the time of activity onset (ca. 30 min) as compared to home cage controls [$n = 8$; $F(1, 107) = 4.17$, $p < 0.05$; Fig. 1]. This difference was significant on postpulse day 1 ($p = 0.005$, *t*-test) and was maintained on most days for up to 10 days after the pulse. Some individuals retained this advanced phase angle for at least 21 days. Individual examples of this effect are shown in Fig. 3.

An additional effect of the nonphotic pulse was to reduce the standard deviation of the onset times. Whereas variability around the mean onset time was similar between experimentals and controls before the pulse, after the pulse there were large differences. Since the standard deviations of both groups were relatively constant after the pulse, we tested 3 days (postpulse days 5, 7, 9) for homogeneity of variance. On all 3 of these days the variation around the mean was significantly different between the two groups ($F > 12.7$, $p < 0.0006$).

There was a significant correlation between the prepulse activity onset time and the magnitude of the day 3 postpulse advance ($r = 0.88$, $p < 0.001$, Pearson Product-Moment Correlation; Fig. 4).

Finally, change in activity time (α), prepulse to postpulse, was compared in pulsed (0.29 ± 0.55 h, mean ± SEM) and home cage control animals (-0.24 ± 0.26 h, mean ± SEM). There was no difference between the groups ($p = 0.89$, Mann–Whitney *U*-test).

DISCUSSION

The results showed that a single exposure to a nonphotic phase-shifting stimulus could alter a hamster's phase angle of entrainment (Ψ). In LD 14:10 the advance induced by a wheel pulse was transient, but in LD 11.5:12.5 the advance lasted at least 3 weeks in some hamsters. Although it is possible to alter Ψ with long-term administration of substances, repeated behavioral manipulations, or by administration of neurotoxins with long-lasting effects (9,10,12,17,22,24,27), no other discrete manipulation that we are aware of has been reported to induce a long-term change in Ψ.

In LD 14:10 the nonphotic pulse advanced the activity onset about 20 min within 2 days after the pulse. Thereafter, activity onset drifted back toward its original prepulse time. The return toward the prepulse Ψ was expected, since one could imagine that the initial phase advance of activity onset was accompanied by an advance of the photic phase response curve (PRC) (3,23). Thus, more light would fall in the delay portion of the photic PRC, forcing a delay in onset time.

The long-lasting change in phase angle in LD 11.5:12.5 is more difficult to explain, but there are at least three ways in which such a shift could have been achieved (18). One possibility is that the nonphotic pulse induced a shortening of the free running period (τ) of the underlying pacemaker, pushing activity

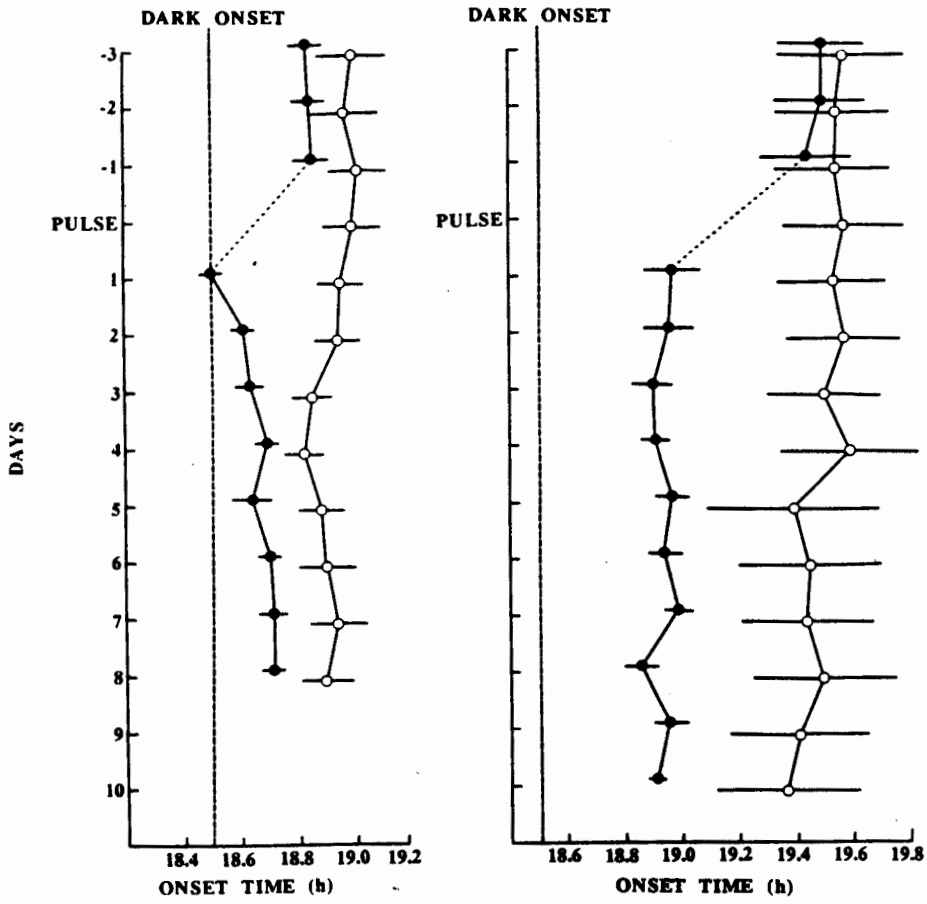

FIG. 1. Mean ± SEM onset times for hamsters for 3 days before and 8 or 10 days after a nonphotic pulse: pulsed animals (●), home cage controls (O). Left panel: animals entrained to LD 14:10 (n = 15, pulsed; n = 5, controls). Right panel: animals entrained to LD 11.5:12.5 (n = 10, pulsed; n = 8, controls).

onset towards lights-off. This seems unlikely, because in other experiments τ of hamsters in constant darkness either lengthens or remains the same after 3-h pulses of novelty-induced running (14).

A second possible way the advanced phase angle could have been achieved is if the nonphotic pulse induced a lengthening of the activity phase (α). Such a lengthening might have extended the advance portion of the hamster's photic PRC, which coincides with the activity phase, past the point of lights-on, thereby inducing a daily advance of the activity onset. In the present experiment (in LD), there was no postpulse lengthening of α in animals that responded with an advance. Two potential problems, however, could weaken the force of this evidence. First, α is difficult to estimate in an objective way, primarily due to the indistinctness and high variability of the end of activity in the hamster. Therefore, there may be some doubt as to whether our arbitrary method of measuring α was accurate. Second, α in the LD conditions of the present study may not have been a good estimate of true α as observed in DD (this may be the critical variable to measure). Lights-on may mask some activity if indeed α is being lengthened by the nonphotic pulse.

A third possible explanation is provided by Turek and Losee-Olsen (25), who demonstrated a similar long-lasting advance of activity onset in hamsters held in a very short photoperiod (LD 6:18) in response to repeated injections of triazolam. They suggested that animals retaining an advanced Ψ after cessation of the injections could really be free running with a period very close to 24 h. Since the night is very long, it might be the case that, after the triazolam-induced phase advance, neither the advance nor the delay portion of the photic PRC was coincident with light. In their study, this seemed to be a real possibility, since the activity records of some individuals did give the impression that the daily activity bout after cessation of triazolam injections was either free running or gradually delaying toward its preinjection phase relationship. The present data for hamsters held on LD 11.5:12.5, on the other hand, showed no tendency for the daily activity bout to return to its original phase relationship after the nonphotically induced advance (at least for up to 21 days postpulse). If the advanced animals were free running, it was with a period extremely close to 24 h.

An additional, less conventional, hypothesis that could also explain a sustained advance in Ψ is that the nonphotic pulse induced a change in the photic PRC. There are a variety of ways the shape of the photic PRC could change to produce the advance in Ψ: a lower amplitude or reduced duration delay portion or a higher amplitude or increased duration advance portion. Any of these changes in the photic PRC would tend to result in a more advanced activity onset relative to lights-off.

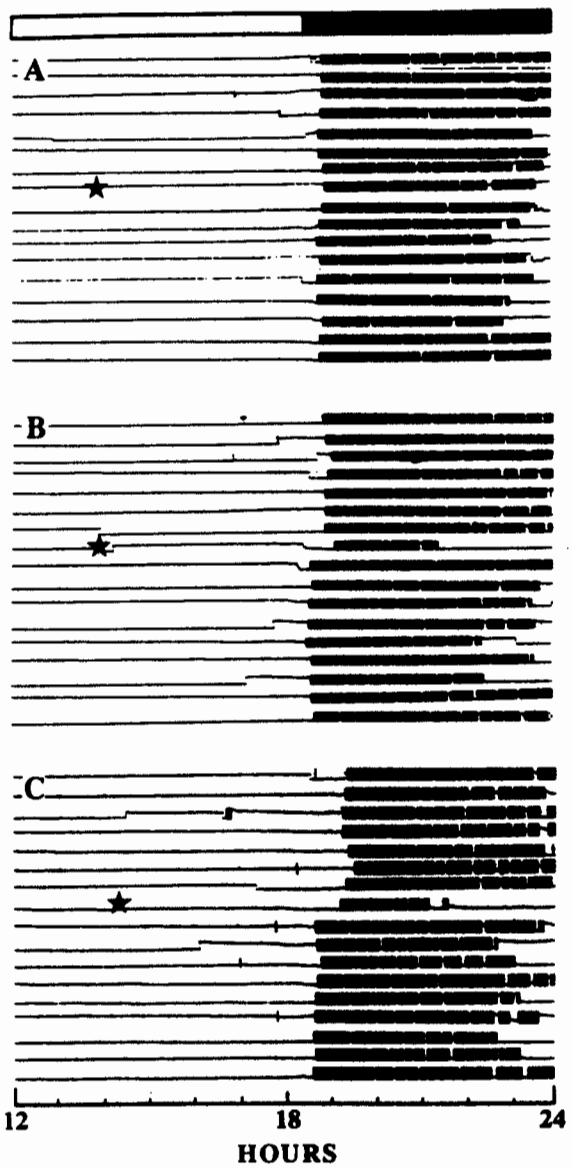

FIG. 2. Running wheel activity records illustrating advances of onset time induced by a nonphotic pulse in LD 14:10. (A) Home cage control; (B) average advance (ca. 20 min); (C) large advance (ca. 40 min). Note that each horizontal span shows 1200 h to 2400 h (0000 h to 1200 h not shown). Consecutive half-days appear one below the other. Light and dark phases are diagrammed at top. Star marks the end of the 3-h nonphotic pulse.

A further aspect of the nonphotically induced shifts in onset in LD 11.5:12.5 was the strong correlation between prepulse Ψ and the magnitude of the postpulse phase shift: the later the activity onset, the larger the phase shift (Fig. 4). This suggests that, whatever the prepulse Ψ in this photoperiod, a nonphotic pulse will advance activity onset to about the same time relative to lights-off. This is also reflected in the reduction of onset variance from prepulse to postpulse. Extrapolation toward the direction of animals with even larger negative phase angles suggests that they would show advances that would bring them in the range of postpulse phase angles observed in the present study. Extrapolation toward smaller, or even positive, phase angles would predict phase delays. However, we would not expect this because the pulses that generated this correlation were given

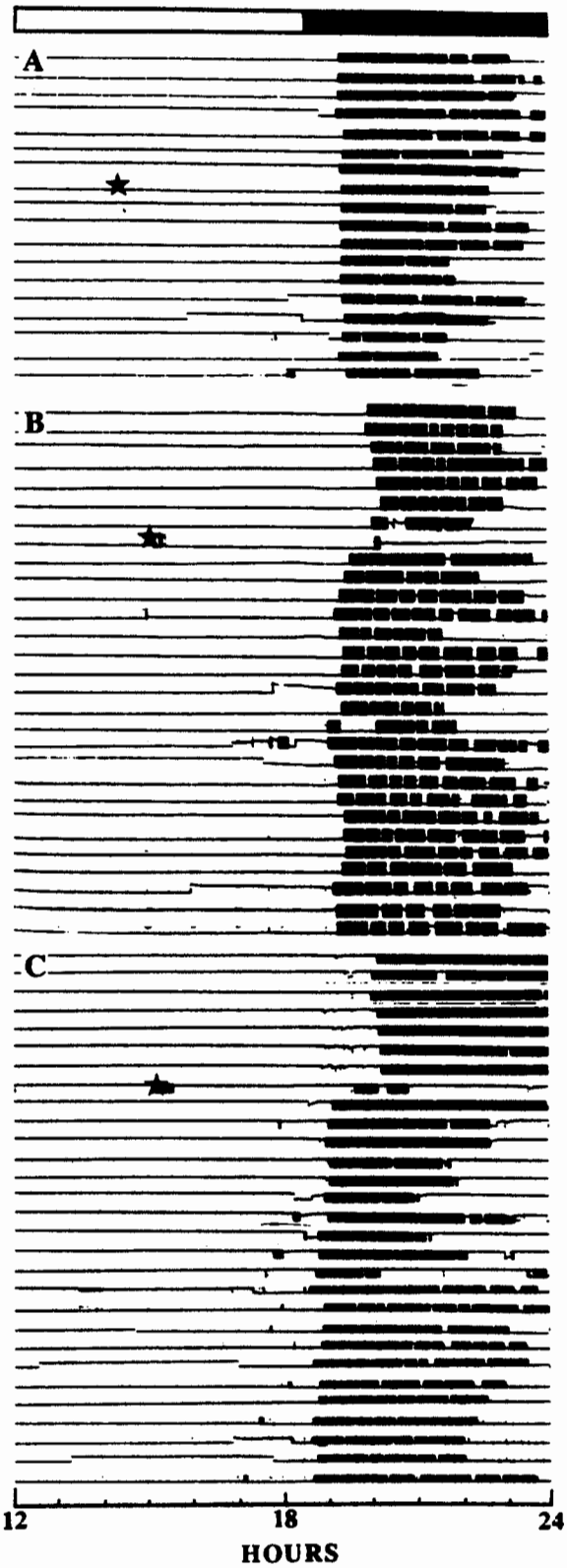

FIG. 3. Running wheel activity records illustrating advances induced by a nonphotic pulse in LD 11.5:12.5. (A) Home cage controls; (B) average advance (ca. 30 min); (C) large advance (ca. 60 min). Note that each horizontal span represents 1200 h to 2400 h (0000 h to 1200 h not shown). Consecutive half-days appear one below the other. Light and dark phases are diagrammed at top. Star marks the end of the 3-h nonphotic pulse.

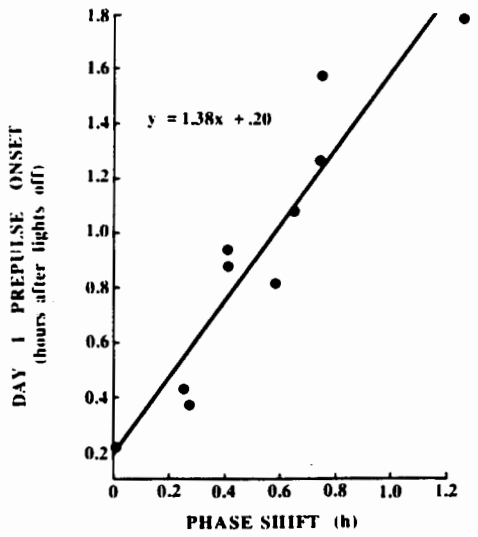

FIG. 4. Relationship between prepulse Ψ and the magnitude of phase advances for hamsters entrained to LD 11.5:12.5. $r = 0.94$, $p < 0.0006$.

during the phase advance portion of the nonphotic PRC. Therefore, we would expect there to be no change in Ψ in response to novelty-induced running for animals with activity onsets earlier than about 0.1 h after lights-off.

If nonphotic stimuli are to be considered as significant factors in the circadian biology of hamsters, they should be able to exert their influence on circadian phase of animals living in a LD cycle. The present results demonstrate that this is indeed possible. Considering the numerous mechanisms for conserving Ψ (18), changes in Ψ of 30 min are notable. Other studies have shown that nonphotic stimuli can alter the phase of entrained rhythms (6,12), but only after repeated daily stimulation. In the present work, a change in phase was achieved after a single nonphotic pulse, indicating how readily nonphotic factors can affect the circadian clock.

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