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Effects of Induced Wheel Running on the Circadian Activity Rhythms of Syrian Hamsters: Entrainment and Phase Response Curve

Stéphan G. Reebs* and N. Mrosovsky*†‡

Departments of *Zoology, †Psychology, and ‡Physiology, University of Toronto, Toronto, Ontario M5S 1A1, Canada

Abstract The goal of this study was to provide an example of nonsocial and nonphotic entrainment in Syrian hamsters, together with a corresponding phase response curve (PRC). Fourteen male hamsters were given 2-hr bouts of induced activity (mostly wheel running) at 23.83-hr intervals in constant darkness (DD). The activity onsets of 10 hamsters entrained to this manipulation, with no anticipatory activity present. After entrainment, the rhythms resumed free-running from a time 0.66–3.91 hr after the onset of the last bout of induced activity. Postentrainment free-running periods were shorter than pre-entrainment values. The PRC for 2-hr pulses of induced activity in DD revealed phase advances induced in some animals between circadian time (CT) 4 and CT 11 (approximately the last half of the hamsters' rest period), and delays between CT 23 and CT 3 and between CT 17 and CT 20. The CTs for phase advances are compatible with the phase angle differences observed between rhythm and zeitgeber at the end of entrainment. Many features of the results (not all animals entraining, PRC characteristics, lack of observable anticipation to the daily stimuli, phase relationship between zeitgeber and activity rhythms) are similar to those from a previous study on social entrainment in this species (Mrosovsky, 1988). These similarities reinforce the idea that induced activity and social zeitgebers act on activity rhythms via a common mechanism.

Social factors can affect the periodicity of activity rhythms in animals kept under constant light (LL) conditions. Evidence for this comes from observations of synchronous free runs (Menaker and Eskin, 1966; Bovet and Oertli, 1974; Wever, 1979; Crowley and Bovet, 1980; Kavaliers, 1980; Regal and Connolly, 1980; Davis and Gorski, 1985; see also Takahashi *et al.*, 1984) and from entrainment studies using cycles of presence-absence of conspecifics (Marimuthu *et al.*, 1981; Viswanathan and Chandrashekaran, 1985; Mrosovsky, 1988) or daily playbacks of conspecific sounds (Gwinner, 1966; Menaker and Eskin, 1966; Reebs, in press) as zeitgebers. Despite this relative abundance of data, the mechanisms whereby social factors affect the period of the internal clock have not yet been determined. Social entrainment can take place in constant darkness (DD; Marimuthu *et al.*, 1981; Reebs, in press); hence we know that the mechanism is nonphotic. A further step is to ask whether the social nature of the stimuli itself is essential for entrainment to take place. Circadian schedules of nonconspecific sounds (Lohmann and Enright, 1967; Eskin, 1971) and cage shaking (Enright, 1975) can entrain activity rhythms. Reebs (in

press) has recently shown that house sparrows entrain to both conspecific and nonconspecific sounds in a similar manner. Furthermore, Mrosovsky (1988) has shown that the phase response curves (PRCs) for social interaction and for cage changing in hamsters are very similar. In several of these studies (Lohmann and Enright, 1967; Mrosovsky, 1988; Reebs, in press), the authors pointed out that the conspecific nature of social zeitgebers may not be essential, and that a common effect of social and nonsocial stimuli may be involved in the mechanism of nonphotic entrainment.

The circadian system of Syrian hamsters, *Mesocricetus auratus*, has been extensively studied. Social entrainment has been obtained in this species (Mrosovsky, 1988). To discover whether similar entrainment could be obtained without cues from conspecifics, we attempted to entrain the activity rhythms of hamsters to a manipulation that was both nonsocial and nonphotic—that is, induced activity (mostly wheel running). We also established the corresponding PRC. Here we report our results.

Entrainment studies are often carried out in LL. This may not be appropriate when studying nonphotic zeitgebers, because photic interferences may confound the results. Such interferences could take place when animals are moved between differently lit parts of the room during manipulations, or when they are awakened and forced to open their eyes, thereby receiving extra stimulation from the light. To avoid this, we conducted our experiments in DD. When appropriate, we establish parallels between our results and photic effects.

MATERIALS AND METHODS

SUBJECTS AND GENERAL PROCEDURES

All hamsters (LAK:LVG, male, 60 days old upon arrival in our laboratory) were obtained from Charles River (Montreal). Once in our laboratory, they were placed in LD 14:10 for 12 days, and then in DD for the experiments proper. Room temperature was $21.0 \pm 2.0^{\circ}$ C. The hamsters were housed singly in one of two cage types: metal-walled cages ($36 \times 20 \times 30$ cm) with a running wheel (17.5 cm diameter) mounted inside; or wire mesh cages ($37 \times 22 \times 19$ cm) without running wheels but with a gnawing bar (Morin, 1978) mounted 2 cm above the cage floor on one side. The running wheels and gnawing bars were connected to an Esterline–Angus event recorder. Food (Purina Chow rodent pellets) and water were available *ad lib*. The cages were replaced with fresh ones every 2–3 weeks, always during the hamsters' normal period of activity; no obvious phase shifts of the activity rhythms were observed following cage change.

To induce activity at specific times, hamsters were moved from their cages into separate running wheels identical to those in the metal-walled cages but from which there was no exit. There they were observed at <4-min intervals and allowed to run, groom, or gnaw at the wheel, without disturbance by the observer. If, however, the animals became motionless or showed signs of sleepiness (curling up and closing the eyes), the observer gently rocked the wheel or blew on the hamsters to induce them to run in the wheel. We used an infrared visionscope (FJW Industries) to observe and

handle the animals. All daily bouts of induced activity lasted 2 hr, after which the hamsters were returned to their home cages.

Actograms were obtained by pasting successive 24-hr activity records below each other. Times of daily onset of activity were measured directly on the actograms. Onsets of gnawing activity were variable and were subjectively defined after visual inspection of the records. Wheel-running activity was more precise, and its onsets were objectively defined as the first 5 min of continuous activity followed by a total of at least 10 min of activity within the subsequent 30 min. In the case of a few animals whose activity diminished toward the end of each experiment, this definition was relaxed to the first 2 min of activity, irrespective of how much activity followed. To prevent using occasional and obvious outliers, a daily onset also had to occur within 1 hr of the previous daily onset.

ENTRAINMENT STUDY

Fourteen hamsters were at first allowed to free-run in DD for 17–29 days. Then, for a subsequent 16–33 days, they were subjected to daily 2-hr bouts of induced activity at a period (T) of 23.83 hr (23 hr, 50 min). The first daily bout was given near the end of the normal rest time for all animals. Following the entrainment schedule, the hamsters were allowed to free-run again for more than 21 days. Activity was recorded with running wheels for nine animals, and with gnawing bars for five animals.

The period (τ) of selected parts of the animals' rhythms was obtained by calculating the slopes of lines that were either eye-fitted through the onsets of gnawing activity, or fitted by least-square regression through the onsets of wheel-running activity. Entrainment (at least that of the activity onset component) was considered to have taken place if the following two criteria were met: (1) The postentrainment rhythm phase, as determined by activity onsets, could not reasonably be predicted by extrapolation of the pre-entrainment rhythm; and (2) both pre- and postentrainment τ 's differed from the zeitgeber T by more than 0.05 hr. A third criterion for entrainment—that of a stable phase relationship between activity rhythm and zeitgeber, even after a phase shift of the zeitgeber (Enright, 1981)—could not be ascertained because the zeitgeber masked activity onset during zeitgeber presentation.

PRC STUDY

In the PRC experiment, single 2-hr pulses of induced activity were given to 20 hamsters (all with running wheels) at various circadian times. Each hamster received four such pulses, in addition to one "sham pulse" (see below). The animals were 90 days old on the day of the first pulse, 107 days old for the second pulse, 124 days old for the third pulse, 144 days old for the sham pulse, and 164 days old for the fourth pulse. Cages were always replaced with fresh ones on a day approximately midway between pulses.

Phase shifts were measured on the actograms by running a first regression line through the last seven definable activity onsets immediately preceding each pulse, and a second one through the seven subsequent onsets (excluding the first onset

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immediately following the pulse). The two regression lines were extrapolated to the day of the first activity onset immediately following the pulse, and the time interval between the two lines was taken as the phase shift. Circadian times (CT) for the onset of the pulse was determined relative to expected onset of activity (CT 12) immediately following the pulse, as given by the extrapolated prepulse regression line. In order to assess the inherent variability of unmanipulated rhythms, "baseline phase shifts" were measured on the day of a "sham pulse," during which no manipulations were performed. A few phase shifts (experimental as well as baseline) could not be measured because of very variable onsets, especially near the end of the experiment. As a result, for the experimental shifts, 2 of the 20 animals gave only two data points, 2 gave three, and 16 gave the full four. Sixteen hamsters gave data points on the day of the sham pulse.

RESULTS

ENTRAINMENT TO T 23.83

None of the 14 hamsters in this experiment showed anticipatory activity before the daily manipulations: They were always found curled up and apparently asleep in their cages at the moment of being transferred to the enclosed wheels. One of five hamsters with gnawing bars and three of nine hamsters with running wheels showed no signs that their rhythms were affected by the entrainment schedule (Fig. 1A). The remaining 10 hamsters fulfilled the two criteria for entrainment. For seven of these hamsters (four with gnawing bars and three with running wheels), the postentrainment rhythm resumed from a time 0.66-1.50 hr after the onset of the last bout of induced activity (Figs. 1B, 1C). Their postentrainment τ 's were always shorter than their pre-entrainment τ 's. These seven hamsters also displayed the same behavior (as observed with the infrared visionscope) once put in the enclosed wheel. They first groomed for about 20 min and then ran vigorously for the remainder of the 2-hr bout (this pattern is very similar to that normally observed in a hamster at the beginning of its subjective night). The last three hamsters (all with running wheels) resumed their postentrainment activity from a time 3.07-3.91 hr after the onset of the last induced activity bout (Fig. 1D). With one partial exception (Fig. 1D), their postentrainment τ 's were shorter than their pre-entrainment τ 's. The behavior of these three hamsters during the bouts was not consistent. None ran vigorously throughout a single bout. Instead, they mostly walked in the wheel, often stopping to sniff or gnaw at the wheel, or to curl up and apparently try to sleep. They often needed prompting to resume walking. The four hamsters that did not entrain also showed this pattern of behavior.

PRC

Figure 2 shows the PRC for the 2-hr pulses of induced activity. A conservative method, based on the range of the baseline values, was used in attributing phase shifts to effects of the manipulation. Phase advances greater than the largest baseline



FIGURE 1. Actograms of four hamsters exposed to 2-hr bouts of induced activity at 23.83-hr intervals (dark slanted lines). Each horizontal line represents 24 hr, and successive days are below each other. Numbers on the right show the calculated rhythm period (τ , in hours) of delimited record segments, with activity onsets used as reference points. (A) Wheel-running activity of a hamster that was not affected by the treatment. (B) Wheel-running activity of a hamster that entrained to the manipulation with a phase angle difference of -1.15 hr between rhythm and induced activity bout at the end of entrainment. (C) Gnawing activity of a hamster that entrained with a phase angle difference of -0.75 hr. On two occasions, no bout could be given (gaps in slanted line). (D) Wheel-running activity of a hamster that entrained with a phase angle difference of -3.07 hr.



FIGURE 2. PRC for 2-hr pulses of induced activity given to hamsters kept in DD. Letters stand in place of data points, with each letter referring to an individual animal. The points to the right are baseline phase shifts measured on intact rhythms; dotted lines show the range of these baseline phase shifts. Underlined letters and points indicate instances where the relaxed criterion was used for activity onset definition (see "Materials and Methods").

value (hereafter called "advances") were obtained only between CT 4 and CT 11 (approximately the last half of the hamsters' rest period). Phase delays greater than the largest baseline value (hereafter called "delays") could be found only between CT 23 and CT 3, and between CT 17 and CT 20. Where advances were obtained, no delays occurred, and vice versa. For example, between CT 4 and CT 11, 12 advances were observed, but no delays (p = 0.0002, binomial test). Conversely, between CT 23 and CT 3, six delays were obtained but no advances (p = 0.0156, binomial test). Some of the advances were 1–2 hr long and readily seen on the records, but almost all delays were much less (Fig. 3).

There were individual differences between animals. For instance, of the 20 hamsters that were tested at least once between CT 4 and CT 11, only 10 gave advances. Of these 10 hamsters, 9 were also tested between CT 23 and CT 3, and 4 gave delays (see H, I, M, and R on Fig. 2). In contrast, some hamsters yielded no advances or delays at any CT (see A, B, D, E, G, and L on Fig. 2). All animals ran well throughout the 2-hr pulses of induced activity in this experiment.

DISCUSSION

Our results show that activity rhythms of hamsters can sometimes be entrained or phase-shifted by a nonsocial and nonphotic manipulation. A feature of the results is



FIGURE 3. Actograms of four hamsters used in the PRC experiment. Closed triangles on the right show days when the 2-hr pulse of induced activity (between dots) was given, with numbers being the calculated phase shift in hours, and question marks denoting instances where no phase shift could be calculated because of diffuse activity. The open triangles denote sham pulse day, during which no pulse was given, but a phase shift was calculated. (A and B) Complete DD records of individuals M and A in Figure 2, respectively. (C and D) Partial DD records of individuals O and C, respectively.

interindividual variability. Some animals appeared totally unaffected by the daily manipulation, whereas others entrained with various phase relationships between activity onset and manipulation onset at the end of the entrainment schedule. Similarly, in the PRC experiment, some individuals did not yield any appreciable phase shifts, but at the same CTs others showed 1- to 2-hr advances. It would be interesting to discover whether the difference between "responders" and "nonresponders" can be related to differential sensitivity to manipulations that are both nonphotic and nonsocial. Differences in behavior like those observed in our first experiment (running vs. walking) may reflect such a differential sensitivity. Alternatively, differences in the neural phase-shifting machinery itself may be responsible. Variability in amplitude of individual PRCs to pulses of light has been previously reported (De-Coursey, 1960), and it would be worthwhile learning whether an individual showing larger-than-average shifts to light would also show larger-than-average shifts to induced activity, or even to social events.

The fact that only some animals responded indicates that our manipulation is a weak zeitgeber. This conclusion is accentuated by two further considerations: (1) Even in responders, phase shifts were relatively small. (2) Periodic bouts of induced activity were not continued for many weeks; this reduced the chances of observing relative coordination or "breakaways" (see Menaker and Eskin, 1966) and distinguishing those phenomena from entrainment. Indeed, the three hamsters whose rhythms exhibited a large phase angle difference with the zeitgeber at the end of the treatment might have shown breakaways had the treatment lasted longer.

Another feature of the results is that entrainment (with $T < \tau$) occurred when the 2-hr induced activity coincided with the late part of the animals' rest period, and no anticipation preceded the bouts. Phase angle differences between activity onsets and zeitgeber varied between -0.66 and -3.91 hr. The location of the advance portion of the PRC (CT 4–11) is consistent with these observations. This suggests that, as is the case for entrainment to light pulses, entrainment to induced activity probably results from small daily phase shifts caused by a stimulus falling on the appropriate part of the PRC. The location of two delay portions on the PRC (CT 17–20 and CT 23–3) leads to the prediction that entrainment by phase delays (T $> \tau$) should also be possible when the bouts of induced activity coincide with the end of the activity period or the beginning of the rest period. However, the small amplitude of the delays on the PRC also suggests that entrainment may take place only when T is close to τ .

After effects on τ following entrainment constitute another feature of our results. After entraining to T 23.83, hamsters showed postentrainment τ 's shorter than their pre-entrainment τ 's. This is striking, because undisturbed hamsters in the conditions of our laboratory have never yet showed a spontaneous decrease in τ during their first 3 months in DD. Similar aftereffects (τ shortening) have been observed in mice after entrainment to light (Pittendrigh and Daan, 1976). This suggests either that the same oscillator and phase-shifting machinery are involved in both photic and nonphotic entrainment, or that similar characteristics are shared if more than one oscillator or phase-shifting machinery is involved.

In a previous experiment in our laboratory, Mrosovsky (1988) entrained the activity rhythms of hamsters free-running in LL with $\tau > 24$ hr to daily (T 24) 1-hr

bouts of social interaction and to daily cage switching (animals being moved into a cage previously occupied by another individual); he also obtained PRCs for 30-min pulses of social interaction and for cage changing (animals receiving a new clean cage). His results are similar to those we present here. In both cases, (1) not all animals entrained; (2) entrainment took place when the zeitgebers coincided with the late part of the hamsters' rest period, with no anticipatory activity preceding the daily bouts; and (3) the PRCs were similar in shape, phase, amplitude, and variability. These similarities support the contention that, in hamsters at least and probably in other species as well (see Reebs, in press), the conspecific nature of social zeit-gebers is essential only inasmuch as it affects the animals in a manner similar to that of induced activity.

This idea leads to the question: What is the nature of the feature that is common to all of these manipulations, and that is involved in the mechanism of entrainment? Although our data leave this question unanswered, they do provide some interesting hints. In our entrainment experiment, separate running wheels were more of a novel stimulus for the hamsters with gnawing bars than for those with running wheels; 80% (4/5) of the former entrained, as opposed to 67% (6/9) of the latter. The difference was not significant (p > 0.05, Fisher's exact test), but the trend suggests that the novelty of a stimulus and the arousal it induces may be important. Similarly, in the same experiment, there appeared to be a correspondence between how active (walking vs. running) an animal was and how closely its activity rhythm entrained to the zeitgeber (as measured by the phase angle difference at the end of the treatment). Running raises body temperature, and this points to the testable idea that elevations in body temperature may have input to the internal clock (see Piercy and Lack, 1988). Finally, we always found the hamsters apparently asleep before the daily manipulations and had to wake them up; moreover, the induced activity that followed is incompatible with normal sleep behavior. Thus it may be that externally provoked disruptions of the normal sleep pattern are part of the mechanism of entrainment. Several techniques exist to reliably deprive animals of sleep (e.g., Tobler and Jaggi, 1987), but to our knowledge these techniques have yet to be used in an entrainment or PRC paradigm. Disentangling the various correlates and consequences of behavioral manipulations and finding out which ones have input to the circadian system is one of the challenges in an attempt to elucidate the mechanisms responsible for nonphotic entrainment.

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