Homeostatic regulation of sleep in arrhythmic Siberian hamsters

Jennie E. Larkin, Tohei Yokogawa, H. Craig Heller, Paul Franken, and Norman F. Ruby*

Department of Biological Sciences, Stanford University, Stanford CA 94305-5020

*Corresponding author:

Norman F. Ruby
Dept. Biological Sciences,
Stanford University
371 Serra Mall
Stanford CA 94305-5020, USA
Phone: +1 (650) 725 6510
Fax: +1 (650) 725 5356
e-mail: **ruby@stanford.edu**

ABSTRACT

Sleep is regulated by independent yet interacting circadian and homeostatic processes. The present study used a novel approach to study sleep homeostasis in the absence of circadian influences by exposing Siberian hamsters to a simple phase delay of the photocycle to make them arrhythmic. Since these hamsters lacked any circadian organization, their sleep homeostasis could be studied in the absence of circadian interactions. Control animals retained circadian rhythmicity after the phase shift and re-entrained to the phase-shifted photocycle. These animals displayed robust daily sleep-wake rhythms with consolidated sleep during the light phase beginning about 1 h after light onset. This marked sleep-wake pattern was circadian in that it persisted in constant darkness. The distribution of sleep in the arrhythmic hamsters over 24 h was similar to that in the light phase of rhythmic animals. Therefore, daily sleep amounts were higher in arrhythmic animals compared to rhythmic ones. During 2- and 6-h sleep deprivations (SD), it was more difficult to keep arrhythmic hamsters awake than it was for rhythmic hamsters. Since the arrhythmic animals obtained more non-rapid eye movement (NREM) sleep during the SD, they showed a diminished compensatory response in NREMS EEG slow-wave activity during recovery sleep. When amounts of sleep during the SD were taken into account, there were no differences in sleep homeostasis between experimental and control hamsters. Thus, loss of circadian control did not alter the homeostatic response to SD. This supports the view that circadian and homeostatic influences on sleep regulation are independent processes.

KEYWORDS

circadian, *Phodopus sungorus*, sleep deprivation, EEG delta power

INTRODUCTION

Sleep is regulated by both homeostatic and circadian processes (3, 6). The homeostatic drive for sleep increases during waking and dissipates during sleep (13, 54). Several aspects of sleep are thought to be homeostatically regulated. Of those, slow-wave activity (SWA; EEG power density in the delta frequency range; i.e., 1-4 Hz) during non rapid-eye-movement sleep (NREMS) most reliably reflects changes in homeostatic drive associated with the sleep-wake distribution (1, 19, 22). Likewise, sleep consolidation and duration co-vary with previous time-spent-awake (17, 20, 21, 34). The circadian system determines the timing of sleep and wakefulness and promotes their consolidation, producing consolidated wake and sleep phases in both nocturnal and diurnal species (6, 14-17). Circadian timing of sleep persists when external time cues, such as the light-dark (LD) cycle, are absent (5).

A variety of approaches have been used to determine the relative contributions of circadian and sleep homeostatic processes to sleep regulation. Lesion studies in animals demonstrated that an intact SCN is not required for the homeostatic regulation of sleep (17, 34, 48, 50). In humans, forced desynchrony protocols established that most aspects of sleep were affected both by sleep-dependent and circadian influences with the exception of SWA. Changes in SWA were found to be driven exclusively by the sleep-wake distribution with little or no circadian contribution (14, 15). These and other observations contributed to the notion that the circadian and the homeostatic regulation of sleep are independent but interacting processes.

Each of these approaches has some potential drawbacks. In a forced desynchrony protocol subjects have to adhere to a longer (or shorter) than normal (i.e., 28- or 20-h instead of 24-h) sleep-wake schedule for multiple days allowing sleep to occur at all circadian phases while time-spent-awake is kept constant. This protocol is challenging to administer especially in animals (47) and relies on mathematically educing the influences of an intact circadian system on sleep. In addition, wake duration is kept constant precluding the direct assessment of the relationship between time-

spent-awake and SWA or sleep duration. Surgical ablation of the suprachiasmatic nuclei (SCN), the central circadian pacemaker in mammals (33), damages adjacent tissue and loss of circadian rhythms has also secondary effects that could potentially confound the results. For example, SCN lesions alter hormone levels (4, 29, 41), that may affect sleep independently of any changes in circadian regulation (e.g. 52). Alternatively, circadian clock function can be disrupted with targeted gene mutations aimed at the canonical circadian clock-genes such as *Clock*, *Bmal1*, *Period*, and *Cryptochrome* genes (37). With this approach loss of gene-function is, however, present in all tissues throughout development, not just in the adult SCN. Furthermore, because most circadian-clock genes are expressed throughout the body (32) and are most likely pleiotropic in function (25), they can be expected to affect other regulatory systems. In fact, studies in transgenic mice and *Drosophila* do suggest that circadian-clock genes may be directly involved in sleep homeostasis independent of their role in generating circadian rhythms (35, 43, 44, 55).

An alternative approach to assess the circadian contribution to sleep homeostasis that does not have the above mentioned drawbacks is to study sleep in intact animals rendered arrhythmic by a phase shift of the LD cycle. Siberian hamsters (*Phodopus sungorus*) can be made arrhythmic by a phase shift of the LD cycle (41) or in response to two consecutive light pulses at night (45), despite continued maintenance in a LD cycle. When hamsters are exposed to a 5-h phase shift of the LD cycle, some retain circadian rhythms and re-entrain to the new LD cycle, some maintain circadian rhythmicity but fail to re-entrain and free-run through the new LD cycle, and some become arrhythmic (41). The latter condition is reversible because rhythmicity is sometimes restored spontaneously or by subjecting hamsters to a period of constant darkness (40, 41). Arrhythmic Siberian hamsters, therefore, provide a unique model system to study homeostatic regulation of sleep in intact animals that lack overt circadian rhythms in physiological measures and behavior (45), especially since both sleep (7-10, 12) and circadian rhythms (23, 24, 38-42) have been intensively studied in this species. We evaluated homeostatic responses to sleep

deprivations of varying duration in animals that either became arrhythmic or maintained rhythmicity and re-entrained to the new LD cycle under the same LD conditions.

MATERIALS AND METHODS

Animal Care and Screening. Siberian hamsters (*Phodopus sungorus sungorus*) were from a breeding colony maintained in our laboratory; the original stock was provided by Irving Zucker (University of California, Berkeley). From birth, hamsters were maintained 3 or 4 to a cage in a 16:8-h LD cycle at 22°C (lights on at 0200 h). Hamsters were provided cotton batting for nesting material; food (Purina chow No. 5015) and water were available *ad libitum*. At 3 months of age, hamsters were housed individually to allow for recording of spontaneous locomotor activity using passive infrared-motion detectors (Model 007.4RTE-A, Visonic, Bloomfield, CT) (41). Activity data were analyzed in 10-min intervals (software by Dataquest, Minneapolis, MN) and analyzed by chi-square periodogram analysis and visual inspection of the actograms (ClockLab, Actimetrics, Evanston, IL) to confirm presence or absence of circadian organization. Entrainment of locomotor activity was verified during the 7 days preceding the phase shift. On the next day, the LD cycle was phase-delayed by 5 h via an extension of the light phase, and activity rhythms were monitored for two months following the phase shift (41). Animals were considered arrhythmic if no significant rhythms were detected by the periodogram analysis and if no LD masking of activity was observed.

Surgery. Entrained (n=6) and arrhythmic (n=5) hamsters were anesthetized with injectable anesthetic [a combination of Ketamine (21.0 mg/ml), Xylazine (2.4 mg/ml), and Acepromazine (0.3 mg/ml)] delivered IP at a dose of 0.34 ml/100g body mass. EEG implant and surgery were as described (20). Briefly, two stainless steel screws were placed through the skull over the right cerebral hemisphere, to serve as EEG electrodes. Two other screws were placed over the left hemisphere and used as anchor screws. Two insulated stainless steel wires inserted into two neck

muscles served as electromyography (EMG) electrodes. The electrodes, which were soldered onto the recording leads before implantation, and the anchor screws were cemented to the skull. The sutured skin was treated with Nitrofurazone powder and animals recovered for two weeks before recordings were initiated.

Sleep Recording Protocol. Sleep was recorded from four hamsters simultaneously, in 7-day recording sessions. During sleep recordings, hamsters were housed individually, given cotton nesting material, and provided with food and water *ad libitum*. Hamsters were held in 16:8 LD cycle during the first 5 days of sleep recording and in constant darkness (DD) during the last two days. Hamsters were connected to recording cables under light halothane vapor anesthesia and allowed to acclimate to the recording conditions for 2 days before sleep recordings began.

Hamsters were connected to a Grass 7 polygraph through a counterbalanced swivel that allowed free movement of the hamster about the cage without undue strain on the skull implant. Unihemispheric, frontal-parietal EEG potentials were filtered (high-pass 0.3 Hz, low-pass 35 Hz; 1/2 max, 6dB/octave), digitized (100 Hz), and stored on a personal computer. The EEG signals were subjected to spectral analysis (Fast-Hartley transformation; 51) over subsequent 10-s epochs. The EMG signals were full-wave rectified, integrated, and stored as one value per 10-s epoch along with the EEG spectra. The original EMG signal was also digitized (at 100 Hz) and stored.

Each recording session consisted of a baseline day, two sleep deprivations (SD; 6 and 2-h in duration; counterbalanced order) each of which was followed by an undisturbed recovery day. The time of lights-on was defined as Zeitgeber Time 0 (ZT0) and lights-off as ZT16. Both SDs ended at ZT6, allowing for comparison of recovery sleep at the same (circadian) time-of-day; i.e. the 6-h SD (SD6) began at ZT0 whereas the 2-h SD (SD2) started at ZT4. Hamsters were sleep deprived by gentle handling. Nesting material was removed and new material added, and hamsters were given food (sunflower seeds and mouse chow) and were occasionally stroked with a soft brush or the side of the cage was tapped to arouse them if necessary. EEG and EMG were recorded

throughout the SD and were used to determine whether hamsters were awake or falling asleep during the SD protocols, along with visual observation of the animals. After the recovery day following the second SD, hamsters were kept in DD for two days. Under DD conditions, sleep recordings from one arrhythmic and two entrained hamsters could no longer be used due to degradation of signal quality and sample sizes reduced to n=4 for both the entrained and arrhythmic group. All procedures were approved by the Stanford University Animal Care and Use Committee.

Analysis of Sleep. The digitized EEG and EMG records were visually inspected, and epochs with artifacts were removed from analysis. Vigilance states were scored based on EEG and EMG signals using an algorithm that has been shown to agree with visual scoring by >90% in mice and in adult and neonatal rats (2, 18, 53). Scoring accuracy of the algorithm was verified by visual inspection. Hourly values were calculated for time spent awake, in rapid-eye-movement sleep (REMS), in non-rapid-eye-movement sleep (NREMS), and mean SWA (0.5 – 4.0 Hz) in NREMS. SWA was normalized to the 24-h mean NREMS SWA in baseline for each animal. NREMS bouts were calculated according to previously published criteria (27). In short, a NREMS bout started with a first 10-s epoch scored as NREMS and lasted until three consecutive 10-s epochs of either wakefulness or REM sleep were encountered.

Statistics. All main effects of factors “arrhythmic/entrained”, “time”, and “condition” (i.e.: baseline, SD2, SD6, and DD) on SWA and vigilance states (NREMS, REMS, wakefulness) were analyzed by one- or two-way ANOVA, with repeated measures where appropriate. Whenever main effects reached significance levels ($P<0.05$), post-hoc Fisher’s PLSD tests were performed to further evaluate differences between the levels of the factors analyzed. Regression analysis quantified the relation between prior sleep time (ZT14 - ZT6) and peak SWA at ZT6, the first hour of recovery sleep following SD. ANCOVA analysis used this covariate (“prior sleep-time”) of SWA at ZT6 against the factor “arrhythmic/entrained”. SWA and vigilance state distributions

during recovery from the 2 and 6-h SDs were compared to each other and to time-matched baseline values, starting at the first hour of recovery following the sleep deprivations (ZT6) using two-sided paired t-tests. SWA values at sleep onset following SD at ZT6 were also compared to SWA at sleep onset under baseline conditions (ZT1).

RESULTS

Hamsters that re-entrained to the new LD-cycle showed robust 24-h rhythms in locomotor activity ($P<0.01$; X^2-test; n=6; Fig. 1). In contrast, no significant circadian rhythmicity could be detected in hamsters that became arrhythmic (n=5, Fig.1). The lack of any activity in the 24-h period range also demonstrates that the LD cycle did not affect or 'mask' locomotor activity in arrhythmic hamsters.

Baseline Sleep under LD conditions: Entrained hamsters slept preferentially during the 16-h light period and were mostly awake during the 8-h dark period (Table 1; Fig.2). The percentage of time spent in NREMS and REMS was significantly higher during the light compared to the dark period (Table 1). Highest amounts of wakefulness were reached during the two hours bracketing light onset (ZT23–ZT1), when hamsters spent 75.5±6.7% of total recording time awake. In contrast, 74.5±1.9% of the remaining 15 h of the light period was spent asleep (Fig.2). The first hour of sleep (starting at ZT1) was characterized by high initial NREMS SWA (130±6%; expressed relative to the mean 24-h baseline value) and a relatively low incidence of REMS (Fig.2). Subsequently, SWA in NREMS decreased and REMS increased and by ZT3 levels typical of the remainder of the sleep period were reached (Fig.2).

In arrhythmic hamsters, the amount of NREMS and REMS did not differ between the light and dark periods and high levels of sleep were maintained throughout the day (Table 1; Fig.2) and, as with locomotor activity (Fig.1), no masking effect of the LD cycle on sleep was apparent. In

entrained hamsters, the consolidation of wake and sleep resulted in a daily rhythm in SWA with highest values at sleep onset (ZT1), in arrhythmic hamsters no daily rhythm in the distribution of vigilance states or in SWA could be observed (Fig.2). Vigilance state distribution was similar for entrained and arrhythmic hamsters during the 16-h light period where both groups of hamsters spent the majority of time asleep (Table 1). NREMS-bout length was similar also during the light period for entrained and arrhythmic hamsters (2.4±0.1 min for arrhythmic and 2.2±0.2 min for entrained hamsters). In contrast, arrhythmic hamsters had significantly longer NREMS bouts during the dark period than entrained hamsters (2.5±0.2 min and 1.8±0.1 min, respectively, $P<0.05$; post-hoc Fisher). NREMS-bout length in the dark period in arrhythmic hamsters did not differ from bout length in the light period of either group ($P>0.05$).

Entrained hamsters showed a strong daily rhythm in REMS, not only in absolute terms (Table 1, Fig.2) but also in its contribution to total sleep time (TST). REMS as a percentage of TST was greater during the second half of the light period than it was in the first half or in the dark period (11.9±0.8% vs. 9.5±0.7% and 9.1±0.6%, respectively; $P<0.05$, post-hoc Fisher). In arrhythmic hamsters, REMS (%TST) did not vary across the day and the average daily percentage (12.5±0.5%) was similar to that reached in the latter half of the light period in entrained hamsters.

Taken together, sleep across the 24-h day in arrhythmic hamsters was similar to that of entrained animals during the light period (Table 1) with the exception of the initial high SWA and low REMS values immediately following the nocturnal active period in the entrained hamsters. Because of the absence of this active period, arrhythmic hamsters spent significantly more time (1.5 h) asleep during the 24-h baseline recording than entrained hamsters (69.4±1.6% vs. 62.8±1.6%, $P<0.05$; Table 1).

Baseline recordings under DD conditions: Recording sleep in DD removes possible direct effects of light ('masking') on sleep in the entrained hamsters. Arrhythmic hamsters remained

arrhythmic in DD, and entrained hamsters retained circadian rhythms in DD (Fig.3). As under LD conditions, in the entrained hamsters, amounts of NREMS were higher during the subjective light period than the subjective dark period (57.6±1.8% vs. 45.2±2.5%, respectively, $P<0.05$), and, conversely, wakefulness was lower during the subjective light period than the subjective dark period (32.8±3.9% vs. 47.5±4.3%, respectively, $P<0.05$). Maximal amounts of wakefulness were again obtained in the two hours immediately preceding the onset of the main sleep period. Sleep onset was characterized by increased NREMS and high initial SWA equivalent to the values obtained under LD conditions. The comparison between sleep under LD and DD conditions did not provide evidence for masking in either arrhythmic or entrained hamsters as vigilance state distribution in the (subjective) light and dark periods did not differ between the two lighting schedules in either entrained or arrhythmic hamsters (Fig.3). In entrained hamsters, SWA levels tended to be higher during the (subjective) light period in the DD condition compared to the LD condition (Fig.3).

Effects of Sleep Deprivation: Sleep deprivation by gentle handling enforced wakefulness in both entrained and arrhythmic hamsters. Some NREMS occurred during the SD but was limited and highly fragmented because animals were awakened as soon as incursions of slow waves in the EEG were observed. Judged by the differences in the amount of NREMS that accumulated over the SDs, it was more difficult to keep arrhythmic hamsters awake than entrained hamsters (2-h SD: 8.4±2.2 vs. 2.9±1.3%, $P<0.05$; 6-h SD: 13.2±2.4 vs. 5.8±1.1%; $P<0.005$, Fig.4). These differences translated into 6 and 26 min more NREMS in arrhythmic hamsters during the 2- and 6-h SD, respectively.

In both groups, 2- and 6-h SDs resulted in increased SWA during the first hour of recovery sleep (ZT6) relative to baseline values at ZT6 ($P<0.001$, Fig.4). Following the 2-h SD the rebound in SWA was short-lasting and in both groups significant deviations from baseline were seen during

the first hour only. In entrained hamsters, SWA values reached after the 2-h SD were equivalent to SWA at ZT1 in baseline (P>0.97, Fig.4); i.e., the time of sleep onset where SWA was highest under baseline conditions. Recovery from the 6-h SD was prolonged in both entrained and arrhythmic hamsters relative to recovery from the 2-h SD, and SWA values were significantly increased for the first 4 h (ZT6–ZT9) relative to baseline values (Fig.4) as well as compared to the levels reached after the 2-h SD (P<0.001), in both groups. NREMS increased and REMS decreased during the first hour of recovery from the 6-h SD (Fig.4). However, neither entrained nor arrhythmic hamsters recovered lost sleep time during the 6-h SD because amounts of both NREMS and REMS did not differ from baseline levels during the 18 h of recovery from the 6-h SD (P>0.53).

Although SWA was significantly increased compared to baseline in the first hour of recovery after SD in both groups, lower values were reached in the arrhythmic hamsters after both 2- and 6-h SD (Fig.4). After the 6-h SD, for arrhythmic hamsters, lower SWA values were also observed in subsequent hours of recovery (i.e., besides ZT6, also significant for ZT8, -11, -14, and -15; P<0.05). The lower SWA values reached in the arrhythmic hamsters might be a consequence of the higher amounts of NREMS observed in this group immediately prior to and during the SD (see above and Fig.4). To account for differences in prior sleep-wake history, the percentage waking during and immediately preceding the SD (i.e., from ZT22 to ZT6) was introduced as a co-factor into an ANCOVA model with factor group ("entrained/arrhythmic") and with the level of SWA reached after the SD as the response variable. The regression of time-spent-awake (between ZT22 and ZT6) against SWA levels reached after the 6-h SD (at ZT6) was significant (P<0.001, R^2=0.73, Fig. 5). This analysis indicates that the decreased wakefulness of the arrhythmic hamsters accounted for the smaller increase in SWA observed following SD. Significant differences in the response to SD were no longer present once the difference in prior wakefulness between entrained and arrhythmic hamsters was accounted for (P>0.21, ANCOVA). Homeostatic

responses to both 2- and 6-h SDs appeared unaltered in arrhythmic hamsters as SWA responded similarly to prior sleep-wake history in both entrained and arrhythmic hamsters (Fig. 5).

DISCUSSION

To separate circadian from homeostatic influences on sleep regulation, we studied the effects of sleep deprivation in anatomically and genetically intact hamsters lacking overt circadian rhythms. Our results confirm the prevailing notion that although circadian processes control the daily distribution and consolidation of sleep and wakefulness, they do not substantially contribute to or modify sleep homeostasis. These conclusions are based on the finding that the relationship between EEG SWA during NREMS and previous time-spent-awake did not differ between arrhythmic and entrained hamsters. EEG SWA in NREMS is a highly predictable homeostatically regulated variable and most of its variance can be explained by the previous sleep-wake history (1, 19, 22). Thus, the level of SWA is high at sleep onset and declines during subsequent sleep according to an exponential function. SWA at sleep onset depends on the preceding duration of spontaneous or enforced wakefulness (10, 19) and intermittent naps will lower SWA at the onset of the subsequent sleep period (13, 54). NREMS intrusions during a SD also lower SWA at recovery sleep onset (19). Prior sleep-wake history had to be accounted for also in the present study because arrhythmic hamsters obtained more sleep immediately prior to the start of the SD and during the SD (Figs. 4,5). If those differences in wakefulness during and immediately preceding the SD had not been taken into account we might have erroneously concluded that the homeostatic regulation of SWA was affected by the absence of an intact circadian system, as SWA levels reached after SD were lower for arrhythmic hamsters (Figs. 4,5). This emphasizes the need to record and quantify sleep obtained during any sleep deprivation protocol.

In concurrence with this study, no change in sleep homeostasis was observed in animals rendered arrhythmic by ablation of the SCN. In the ablation studies, SD was followed by

increased SWA and a higher prevalence of deeper stages of NREMS that were comparable to intact animals (17, 48, 50). Thus, our results suggest that the secondary effects of lesions aimed at the SCN (see Introduction) do not seem to significantly affect the homeostatic regulation of sleep.

One of the more prominent differences between the two groups of hamsters was found in baseline conditions where arrhythmic hamsters slept ~1.5 h more than rhythmic ones over a 24 h period. Together with the findings that NREMS in arrhythmic hamsters was, on average, more consolidated and that they were more difficult to keep awake during SD, these observations could be taken as evidence that sleep need is increased in the absence of circadian rhythmicity. However, when challenged with a SD, the homeostatic response in SWA and NREMS amount during recovery did not differ between the two groups of hamsters. This indicates that by itself the amount of sleep obtained under baseline conditions does not reflect the level at which sleep is homeostatically regulated. In squirrel monkeys rendered arrhythmic by lesioning the SCN (SCNx) increases in sleep time have also been observed (17). These results gave rise to the view that a SCN-dependent process actively facilitates the initiation and maintenance of wakefulness specifically (16, 17). Increases in sleep time in SCNx animals can thus be seen as a mere consequence of a lack of wake promotion. Lesioning the SCN in rats and mice is, however, not accompanied by an overall change in sleep duration (26, 34, 48, 50).

In intact animals, sleep need that accumulates during the nocturnal period of consolidated wakefulness is manifested early in the subsequent rest period by initial high levels of SWA and NREMS and by increased NREMS consolidation. As SCNx animals lack a distinct active period, high levels of sleep need are never reached under baseline conditions. As a consequence, SWA and sleep consolidation are, on average, lower in SCNx mice and rats (26, 34, 48, 50) and lighter stages of NREMS are more prevalent than in intact animals (17). Because sleep amount and consolidation in arrhythmic hamsters remained similar to daytime consolidated sleep in entrained hamsters, mean daily values for sleep amount and consolidation were significantly greater in

arrhythmic than entrained hamsters. Thus, sleep in arrhythmic hamsters differed from that in other rodents made arrhythmic through SCN lesions, in that mean 24 values of sleep duration and NREMS consolidation was increased relative to controls. It remains to be determined, however, whether SCN lesions affect sleep in Siberian hamsters as they do in rats and mice to rule out potential species differences.

It should be emphasized that the SCN remains intact in arrhythmic hamsters, although we assume that it no longer provides rhythmic output. Output from the SCN is thought to directly drive the daily/circadian patterns in sleep-wake consolidation (3, 5, 6, 16, 17). Both *in vitro* and *in vivo* recordings demonstrate that the daily changes in spontaneous neuronal activity within the SCN correlate well with the daily changes in locomotor activity and sleep; e.g. in nocturnal rodents a high level of spontaneous SCN activity is associated with consolidated periods of inactivity and sleep during the rest phase (11, 30, 31, 56). Because sleep amount and consolidation in our arrhythmic hamsters were maintained at the levels reached during the rest phase in entrained hamsters, one could hypothesize that SCN activity should therefore also be maintained at the high levels typical for the rest period. If so, this may account for the increased duration and consolidation of NREM sleep observed in arrhythmic hamsters in the present study compared to the more fragmented sleep measured in SCNx mice and rats that lack SCN output altogether.

Siberian hamsters housed in short daylengths lose circadian organization in sleep, but that model of arrhythmicity is not directly comparable to the present one. Daylength affects the distribution and consolidation of sleep and wakefulness and may induce arrhythmia in some sleep parameters. Siberian hamsters kept in short day-lengths and at low ambient temperature display reduced amplitude or arrhythmia in diurnal rhythms of sleep time and SWA (8, 9, 12). Despite the observed changes in sleep-wake distribution between short and long day-length conditions, daily sleep duration did not vary. Disruption or loss of circadian sleep rhythms induced by short daylengths does, however, not induce arrhythmia in other circadian rhythms. Siberian hamsters

maintain coherent circadian rhythms in body temperature, melatonin, and locomotor activity despite loss of circadian sleep rhythms (8, 28). Hamsters housed in short daylengths also undergo dramatic changes in their physiology that sharply differentiate them from long-day hamsters. Thus, sleep in arrhythmic hamsters in the present study cannot be directly compared to sleep in hamsters maintained in short day-lengths that are partially arrhythmic because the hamsters in the present study are arrhythmic in all physiological measures determined thus far; i.e., sleep, body temperature, melatonin, and locomotor activity (41, 45) and therefore provide a good alternative to SCN-lesioned animal model.

The lack of modulation in the daily distribution of both activity and sleep in arrhythmic hamsters by the light-dark alternation (Figs.1,2) together with the absence of a significant effect on the distribution of sleep under constant dark (DD) conditions in entrained hamsters (Fig.3) demonstrate that ambient light does not directly affect these behaviors. In contrast, EEG SWA within NREMS during the subjective light period under DD seemed to be elevated compared to the levels reached during the prior light period. Similar observations have been made in rats (49) and are likely to reflect the direct inhibitory effect of ambient light on delta (1-4 Hz) oscillations of thalamocortical origin (36) thought to underlie EEG SWA (46).

Perspectives: Our findings underscore the notion that although circadian regulatory processes are key in timing sleep at the appropriate time-of-day, they do not seem to influence homeostatic regulatory aspects of sleep. Because of their unusual responses to shifts in the LD cycle, Siberian hamster may provide a unique model system to further investigate mechanisms of circadian rhythm generation, entrainment to external stimuli, and coordination of circadian rhythms and other biological processes such as sleep.

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Table 1. Comparison of amount of sleep between arrhythmic hamsters (n=5) and rhythmic hamsters that re-entrained to a 16:8 LD-cycle (n=6). Data presented as mean (SE) percentage artifact-free recording time. [*]indicates a significant difference ($P<0.05$) between light (L) and dark (D) values within each group. [†]indicates a significant difference ($P<0.05$; post-hoc Fisher) between the two groups.

		Entrained	**Arrhythmic**
Waking	L (16h)	28.6 (2.1)	30.0 (2.1)
	D (8h)	54.5 (3.2)[*]	31.9 (2.4)[†]
	24h	37.2 (2.0)	30.6 (1.6)[†]
NREMS	L	63.6 (1.8)	61.2 (2.0)
	D	41.4 (2.9)[*]	59.8 (2.2)[†]
	24h	56.2 (1.8)	60.7 (1.5)[†]
REMS	L	7.8 (0.5)	8.9 (0.5)
	D	4.1 (0.4)[*]	8.3 (0.6)[†]
	24h	6.6 (0.4)	8.7 (0.4)[†]

FIGURES

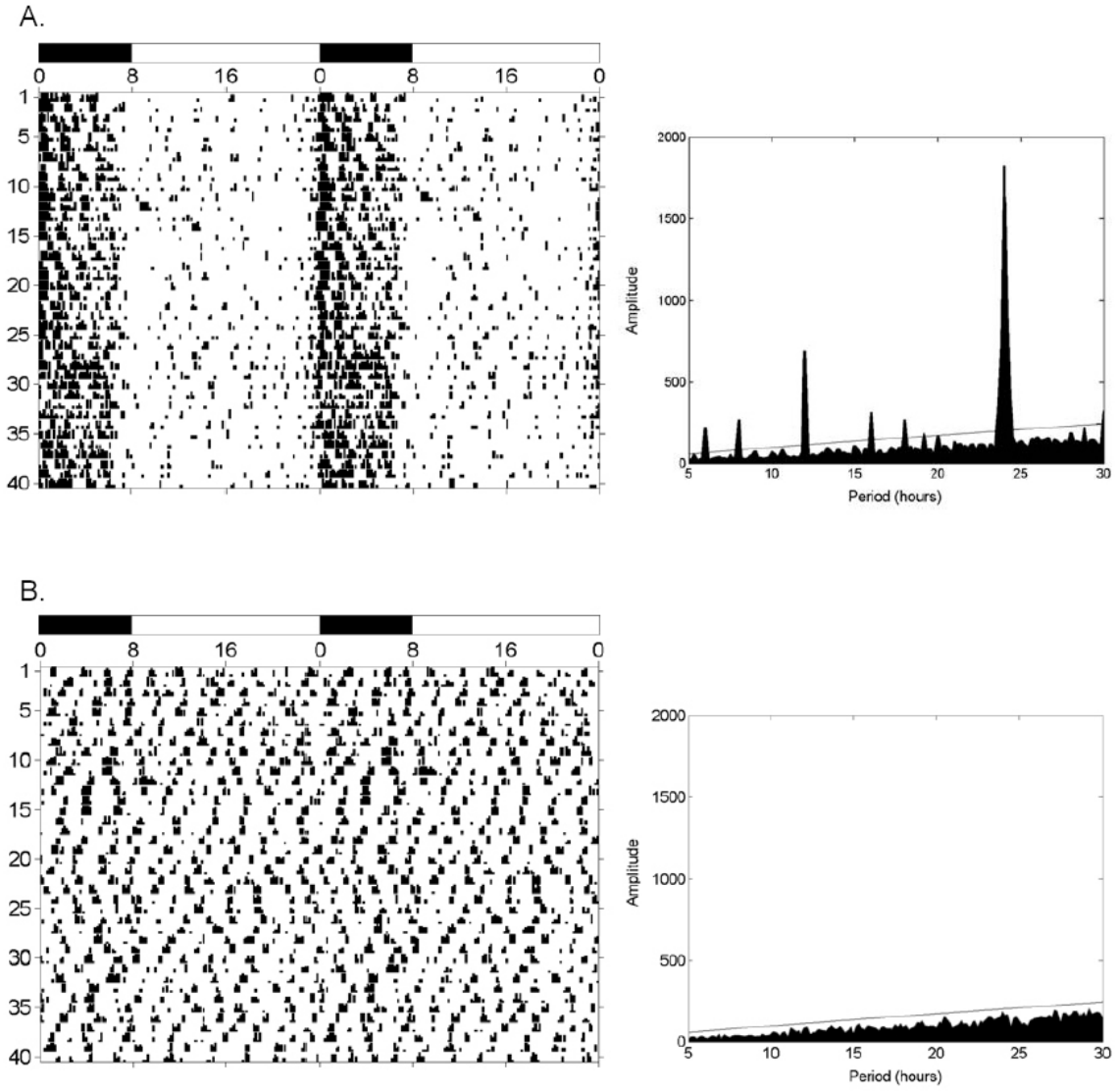

Figure 1: Double-plotted locomotor activity data for 40 days (left panels) and X^2-periodogram analysis (right panels) of a hamster that remained rhythmic and re-entrained to a 5-h phase delay in the 16:8 LD-cycle (A) and of a hamster that became arrhythmic (B) after the phase shift. LD cycle is indicated by the horizontal bars. Straight lines in the periodograms indicate the P=0.01 significance level (X^2-test).

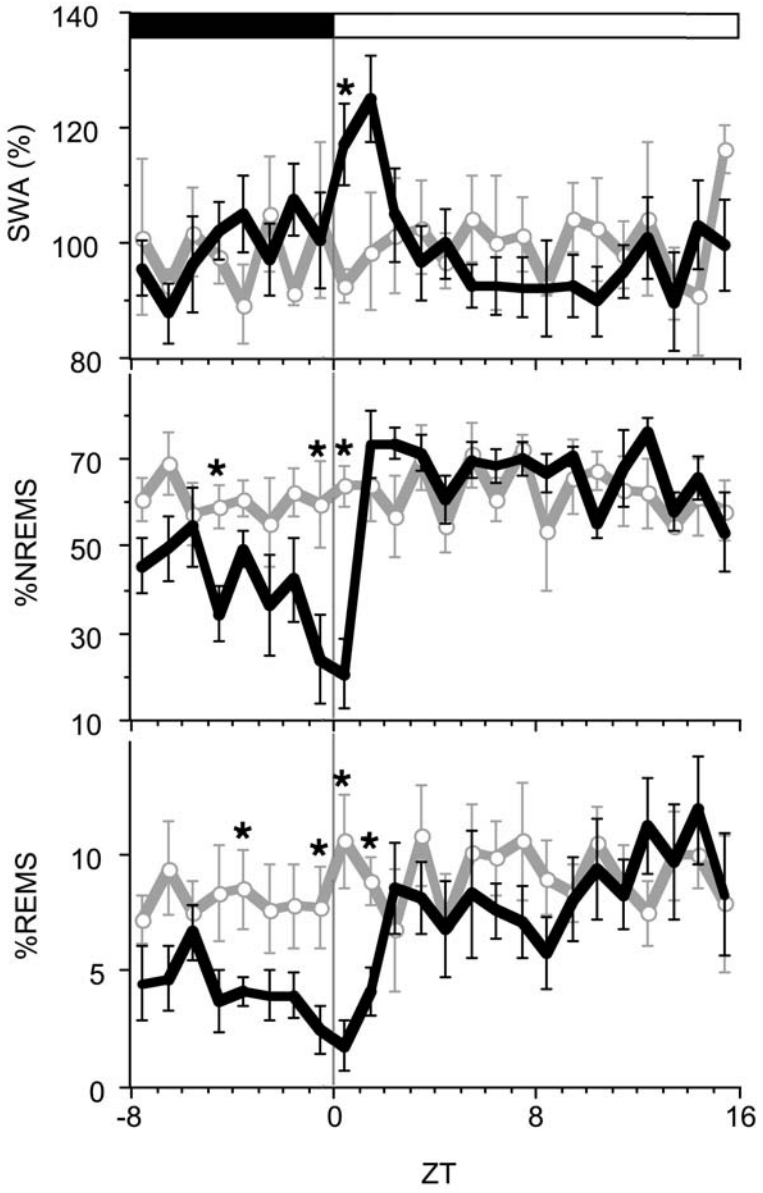

Figure 2: Time course of SWA and sleep duration in entrained (black lines, n=6) and arrhythmic (grey lines, n=5) hamsters in baseline. Values indicate mean ± SE hourly values. Significant differences ($P<0.05$; post-hoc Fisher) between arrhythmic and entrained hamsters are indicated by *. Values for sleep are expressed as % of artifact recording time, for SWA as % of individual mean level of SWA in NREMS over 24-h baseline (=100%). The same mean SWA levels served as a reference for the analysis presented in Figs. 3, 4, and 5. The 16:8 LD cycle is indicated on top by the open and filled horizontal bars, respectively. ZT0 is the time of light-onset.

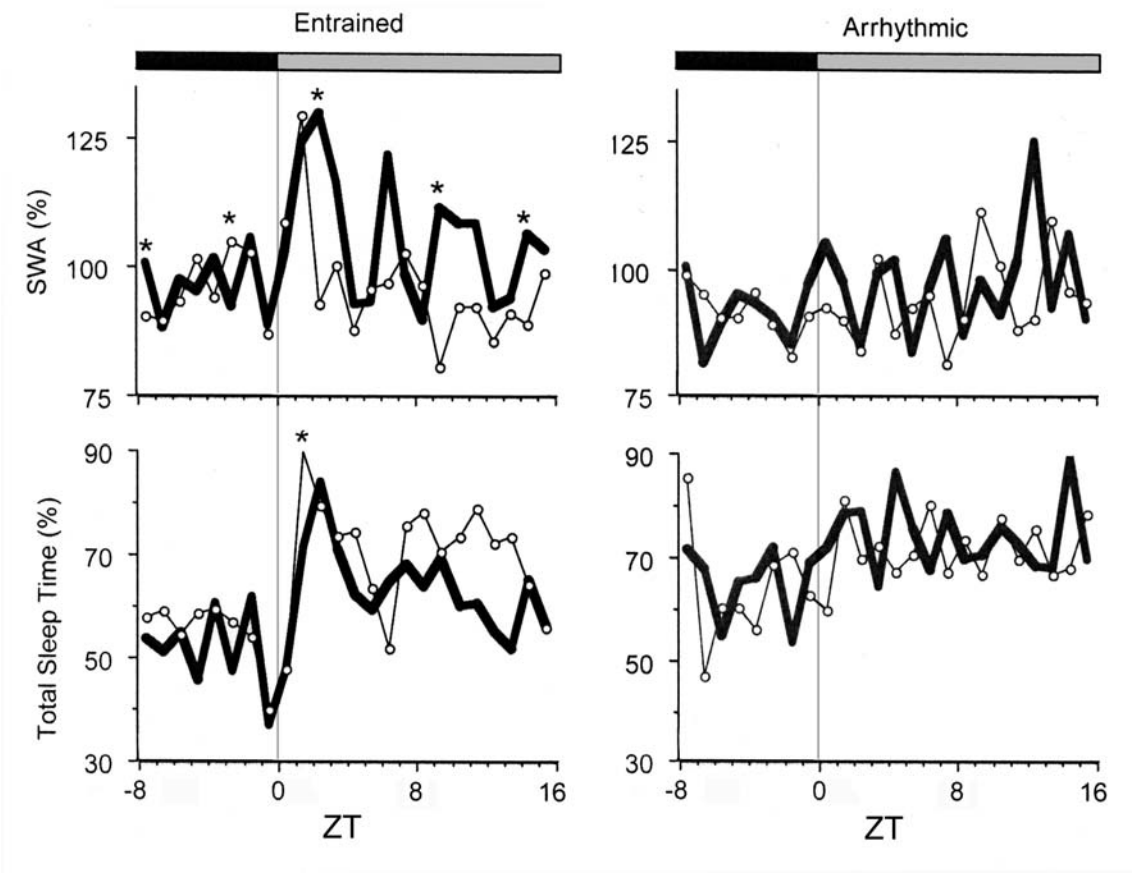

Figure 3: Time course of SWA and total sleep time (% recording time) in entrained (left, n=4) and arrhythmic (right, n=4) hamsters during the last day in 16:8 LD cycle (thinner lines) and the first day in DD (thicker lines). * indicate significant differences between LD and DD conditions for each group ($P<0.05$; post-hoc paired t-tests). For details see Fig.2. The (subjective) 16:8 rest-active (i.e. LD) period is indicated on top by the grey and black horizontal bars, respectively.

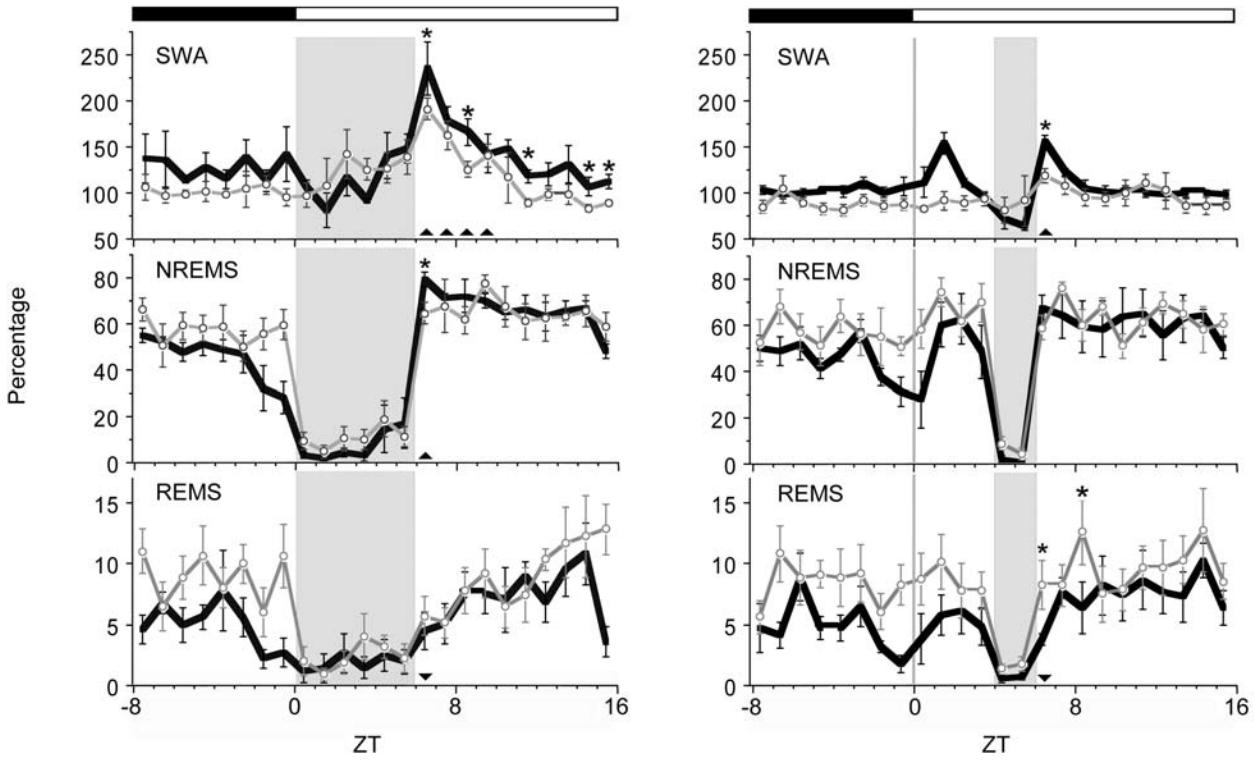

Figure 4: Time course of SWA and sleep duration in entrained (black lines, n=6) and arrhythmic (gray lines, n=5) hamsters following a 6-h (ZT0-ZT6; left panels) and a 2-h (ZT4-ZT6; right panels) SD. SD periods are indicated by gray areas. * indicates significant differences between groups (P<0.05; post-hoc Fisher) and are indicated only for the recovery period (for baseline differences see Fig.2). ▲ and ▼ indicate intervals in which values significantly increased or decreased, respectively, from corresponding baseline values (P<0.05; post-hoc paired t-tests). Deviations were the same for both groups. For details see Fig.2.

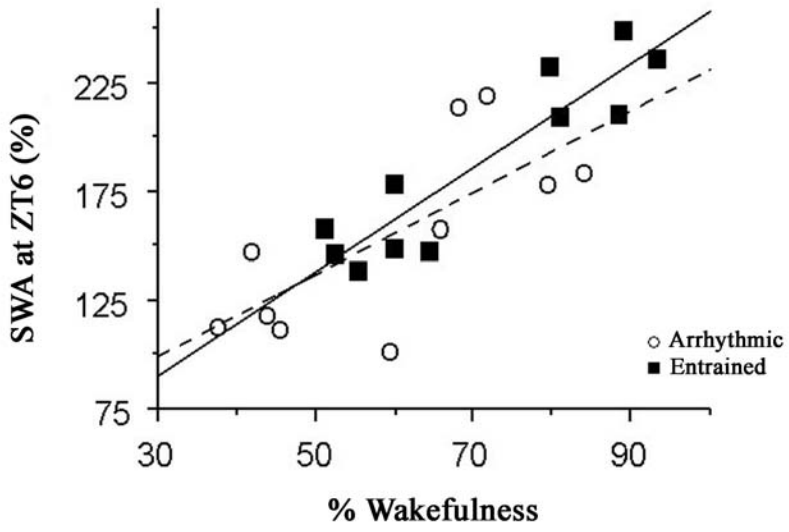

Figure 5: Regression of SWA during the first hour (ZT6-ZT7) of recovery following 2- and 6-h SDs against mean level of wakefulness during the 8 preceding hours (ZT22–ZT6). Entrained hamsters indicated by black squares (■), arrhythmic hamsters by open circles (○). The regression was significant (P<0.0001, for both groups combined: R^2=0.73, n=22; arrhythmic: R^2=0.52, n=10; entrained: R^2=0.84, n=12). Slopes of the regression lines for entrained (solid line) and arrhythmic (dashed line) hamsters were similar (arrhythmic: SWA=1.9·%wakefulness + 42.4: entrained: SWA=2.4·%wakefulness + 18.6).