

Feeding Schedule Controls Circadian Timing of Daily Torpor in SCN-Ablated Siberian Hamsters

Matthew J. Paul,^{*,1} Alexander S. Kauffman,[†] and Irving Zucker^{*,†}

Departments of ^{*}Psychology and [†]Integrative Biology, University of California at Berkeley, Berkeley, CA

Abstract Timing of daily torpor was assessed in suprachiasmatic nucleus-ablated (SCNx) and sham-ablated Siberian hamsters fed restricted amounts of food each day either in the light or dark phase of a 14:10 light-dark cycle. Eighty-five percent of sham-ablated and 45% of SCNx hamsters displayed a preferred hour for torpor onset. In each group, time of torpor onset was not random but occurred at a mean hour that differed significantly from chance. Time of food presentation almost completely accounted for the timing of torpor onset in SCNx animals and significantly affected timing of this behavior in intact hamsters. These results suggest that the circadian pacemaker in the SCN controls the time of torpor onset indirectly by affecting timing of food intake, rather than by, or in addition to, direct neural and humoral outputs to relevant target tissues.

Key words torpor, SCN, hamster, food restriction, circadian, food anticipatory activity

During daily torpor, several rodent species decrease body temperature (T_b) from 37 °C to ~20 °C for several hours (Hill, 1975; Hudson, 1978). The accompanying reductions in metabolic activity and many physiological and behavioral processes reduce daily energy expenditure (Geiser, 1988; Heldmaier and Ruf, 1992; Ruf and Heldmaier, 1992). Torpor can be elicited by food restriction or decreases in day length (Morhardt and Hudson, 1966; Lynch et al., 1980; Heldmaier and Steinlechner, 1981; Elliott et al., 1987), both of which are associated with weight loss and reduced food availability.

Torpor usually occurs 2 to 3 times per week in Siberian hamsters housed in winter day lengths and low ambient temperatures; torpor onset is restricted to the early rest phase, with rewarming initiated prior to onset of the normal active phase (Ruf et al., 1989). In common with other circadian rhythms, onset of daily torpor free runs when animals are kept in constant darkness (Ruf et al., 1989) and phase shifts over the

course of several days after a shift in the light-dark (LD) cycle (Kirsch et al., 1991). Ablation of the SCN circadian pacemaker eliminates spontaneous daily torpor (Ruby et al., 1989); a brief interval of postoperative food restriction reinstates torpor bouts, yet its expression is no longer rhythmic (Ruby and Zucker, 1992). This suggested that torpor is a circadian behavior whose timing is under the control of the SCN circadian pacemaker and entrained to the LD cycle.

The discovery of rhythmic clock gene expression in nonneural tissues has led to changed conceptions of circadian organization. The SCN is no longer viewed as monolithically imposing rhythmicity on nonrhythmic tissues but rather as synchronizing endogenous oscillators located throughout the body (Sakamoto et al., 1998; Yamazaki et al., 2000; Allen et al., 2001; Wakamatsu et al., 2001), perhaps by controlling feeding behavior (Le Minh et al., 2001; Stokkan et al., 2001). In mice forced to consume their food during the daily rest phase, the expression of

1. To whom all correspondence should be addressed: Matthew J. Paul, Department of Psychology, University of California, Berkeley, Berkeley, CA 94720-1650; e-mail: mattp@socrates.berkeley.edu.

clock genes in non-SCN tissues shifts by approximately 180°, while clock gene expression in the SCN is not altered (Damiola et al., 2000; Le Minh et al., 2001; Stokkan et al., 2001; Wakamatsu et al., 2001). Because the SCN normally determines when animals eat (Strubbe et al., 1987; Cipolla-Neto et al., 1988), it may convey circadian information to oscillators indirectly via its control over feeding patterns instead of, or in addition to, direct neural and humoral communication with target tissues.

There are striking similarities between peripheral clock gene expression after food restriction and food-related anticipatory activity (FAA). FAA, defined as increased locomotor activity immediately prior to a scheduled feeding, emerges in animals placed on a food restriction regimen (Stephan, 2002). FAA displays several circadian characteristics (Boulos et al., 1980; Stephan, 1981, 1984) and may be controlled by the same mechanism that influences extra-SCN gene rhythms. FAA and non-SCN gene expression both dissociate from the SCN circadian pacemaker and follow the feeding schedule (Stephan, 1986; Damiola et al., 2000).

The timing of food presentation also influences the onset of torpor in food-restricted Siberian hamsters (Bae, 2000). Food-restricted Siberian hamsters maintained on an 8:16 LD cycle and presented with food at zeitgeber time (ZT) 16 and 20 enter torpor later in the photocycle than hamsters fed at ZT 7 and 12. This suggests that timing of torpor onset is determined by the circadian pacemaker's control over eating patterns, rather than by direct SCN control. If this conjecture has merit, then the torpor onset rhythm should be reinstated in SCNx animals maintained on a restricted feeding regimen, and timing of torpor should be determined by the time of food presentation, not the daily LD cycle. We tested these predictions and provide evidence that the timing of food intake exerts an important influence on the timing of torpor onset.

METHODS

Animals and Housing Conditions

A total of 147 adult female Siberian hamsters (*Phodopus sungorus*) were maintained from birth in an LD 14:10 photocycle (14 h light/day; lights on at 0400 h PST) at 23 °C and group housed in polypropylene cages (25 × 14 × 12 cm). Food (Purina rodent chow

5015) and water were available ad lib unless otherwise noted. At the start of the experiment animals were singly housed and transferred to a cold room maintained at 5 °C with the original 14:10 LD photocycle.

Food Restriction

Mean daily food intake was calculated for each animal by measuring food consumption over 24-h intervals for 7 consecutive days. Food intake was initially lowered to 90% of the baseline value and subsequently decreased by 5% decrements. Body mass was monitored and determined the rate of food intake decrements. No animal was given less than 50% of its baseline food intake, and food restriction was stabilized for each animal upon the occurrence of the first torpor bout. No animal was food restricted for more than 11 weeks during the torpor screen and 8 weeks during the second interval of food restriction. Because food pellets absorb moisture at low ambient temperatures, they were maintained at 5 °C for at least 1 week before use. Automatic feeders connected to a timer dispensed food pellets onto the animal's cage lid at the desired time. This allowed animals to be fed without the experimenter entering the room and eliminated external time cues associated with feeding.

Surgeries

Surgeries were performed under deep anesthesia induced by administering a ketamine cocktail (21 mg ketamine, 2.4 mg xylazine, and 0.3 mg acepromazine/mL injected i.p. in a dose of 0.34 mL per 100 g body mass). Bilateral radio-frequency lesions aimed at the SCN were made with a Radionics Model RFG-4 A Research RF Lesion Generator system (Radionics, Burlington, MA; coordinates: 0.0 mm anterior to bregma, 0.2 mm lateral to the midline, and 6.3 mm below dura, with the head leveled between bregma and lambda). Current was delivered to raise the electrode tip temperature to 80 °C for 30 sec per lesion. During sham surgeries, the electrode was lowered 4.5 mm below dura, without passage of current. In 47 of 62 animals, radio-frequency transmitters were implanted intra-abdominally via a single midline incision immediately after brain surgery. For the remaining 15 animals, the transmitter was implanted 4 weeks after the brain lesion procedure. Postsurgical analgesia was induced by an s.c. injection of buprenorphine (0.15% in 0.1-mL vehicle).

Telemetric Recording of T_b and Locomotor Activity

Calibrated temperature-sensitive radio-frequency transmitters (model VM-FH, MiniMitter, Sunriver, OR) for recording T_b and locomotor activity were implanted intra-abdominally. Signals from the transmitters were collected every 10 min via receiver boards placed beneath each animal's cage and subsequently processed by an automated computer program (Dataquest, St. Paul, MN).

T_b and Locomotor Activity Analysis

Rhythmicity of general locomotor activity and T_b data was determined by visual and periodogram analysis. During the experimental period, torpor bouts were identified by visual inspection of T_b data. To qualify as a torpor bout, T_b had to be maintained at $< 31^\circ\text{C}$ for at least 30 min. Duration of torpor was defined as the interval during which T_b was $< 31^\circ\text{C}$; torpor onset was indicated by the initial decline of T_b to $< 31^\circ\text{C}$ and torpor offset by the time at which T_b was first $> 31^\circ\text{C}$. $T_{b\text{min}}$ was defined as the lowest T_b during the torpor bout. Hamsters often displayed multiple torpor bouts in one 24-h period. The data were analyzed twice, once using the 1st torpor bout after midnight and a 2nd time using the first torpor bout after feeding as the representative bout. There were no differences in the group comparisons between these 2 statistical analyses, and only the latter is reported in this study so that torpor onsets reflect true latencies from feeding time. To determine the occurrence of feeding-induced anticipatory activity (FAA), the percentage of daily locomotor activity during the 3-h interval preceding food presentation was determined for the 4 days before and the day of the 4th torpor bout; these values were compared to the daily percent activity of the same 3-h interval during the last 5 days of ad lib feeding. FAA was said to have occurred if the daily activity in the 3 h prior to food presentation was $\geq 200\%$ of the corresponding value during ad lib feeding.

Torpor Screen

Hamsters 3 to 7 months of age were screened for daily torpor in response to food restriction before any experimental manipulation. Food was presented at 1500 h, and visual inspections for torpor were conducted daily at 0700, 0900, and 1100 h. Torpor was

assessed according to the following criteria: sluggishness, slumped posture, nonresponsiveness to a dangling object, and nonresponsiveness to touch (Bae, 2000). Hamsters that displayed ≥ 3 of these behaviors were considered torpid. Hamsters that displayed at least 2 torpor bouts during 7 consecutive days were divided into 2 groups, designated for bilateral lesions of the SCN (SCNx; $n = 44$) or sham surgeries (Sham; $n = 18$). Animals recovered from surgery at 23°C for at least 1 day before they were returned to 5°C . Food was again provided ad lib until each hamster's body mass returned to baseline values. After recovery from the final surgery, locomotor activity was recorded and the data were analyzed to confirm arrhythmicity in the SCNx group (Ruby and Zucker, 1992). SCNx animals with evidence of residual circadian rhythms in their locomotor activity records were excluded from further analyses.

Experimental Procedure

Upon full recovery from surgeries and verification of circadian status, an experimental period of food restriction was enforced, during which some animals from each group were presented with food at 0700 h (AM-Sham and AM-SCNx) and the remainder at 1900 h (PM-Sham and PM-SCNx). Locomotor activity and T_b were recorded continuously, and the occurrence of torpor was determined by analysis of T_b records. Animals that displayed 4 torpor bouts on at least as many different days were considered to have completed the study and were sacrificed for brain histology. Body mass was recorded 1 to 3 times/week throughout the study. Locomotor activity records were analyzed for the presence of FAA.

Histology

Hamsters were administered a lethal dose of sodium pentobarbital and perfused transcardially with 0.9% NaCl (20 mL) followed by 10% formalin in phosphate-buffered saline (20 mL). Brains were removed and placed in 15% sucrose/10% formalin/phosphate-buffered saline for a minimum of 24 h. Following fixation, brains were sliced on a freezing microtome at $40\ \mu\text{m}$ and stained with cresyl violet for microscopic analysis.

Experimental procedures were approved by the Animal Care and Use Committee, University of California, Berkeley.

Statistical Analysis

Differences in FAA and torpor characteristics other than torpor onset were analyzed with ANOVA (Statview 5.0; Abacus Concepts, Berkeley, CA). For each hamster, the mean value from 4 torpor bouts was used in analyzing group differences. Timing of torpor onset relative to clock time and time of food presentation was analyzed with circular statistics (Batschelet, 1981). Clustering of torpor onsets to 1 time of day was determined separately for each animal using the Rayleigh test (Stephens, 1969; Batschelet, 1981). Second-order analysis of torpor onsets was performed with Hotelling's 1-sample test to determine whether torpor onset vectors within each group were clustered at 1 time of day (Batschelet, 1981). Individual hamsters and groups that met clustering criteria were considered to have a preferred torpor onset time. Planned group comparisons were assessed with Hotelling's 2-sample test. Differences in percentages between groups were evaluated with chi-square tests.

One animal was inadvertently removed from the study after displaying 3 instead of 4 torpor bouts (PM-SCNx group). Data for this animal were included in the statistical analyses to increase the sample size.

RESULTS

Of the animals, 62 of 147 successfully passed the initial torpor screening test and underwent surgery. Fourteen hamsters with lesions that targeted the SCN continued to display rhythmic circadian locomotor activity after SCN_x, and 1 sham-ablated animal displayed a free-running rhythm. These animals were eliminated from further consideration; an additional 11 hamsters were excluded because of intraoperative death or poor postoperative health. This left 21 arrhythmic SCN_x and 16 rhythmic sham-ablated animals for designation for AM or PM feeding. Thirteen of these animals failed to display 3 torpor bouts and were thus excluded from further analyses (0, 5, 3, and 5 hamsters for AM-Sham, AM-SCNx, PM-Sham, and PM-SCNx groups, respectively). There was not a selective bias among groups associated with elimination of hamsters that failed to meet the torpor criterion ($p > 0.05$). Based on these exclusions, the final sample sizes for each group were as follows: AM-Sham ($n = 6$), AM-SCNx ($n = 5$), PM-Sham ($n = 7$), and PM-SCNx ($n = 6$).

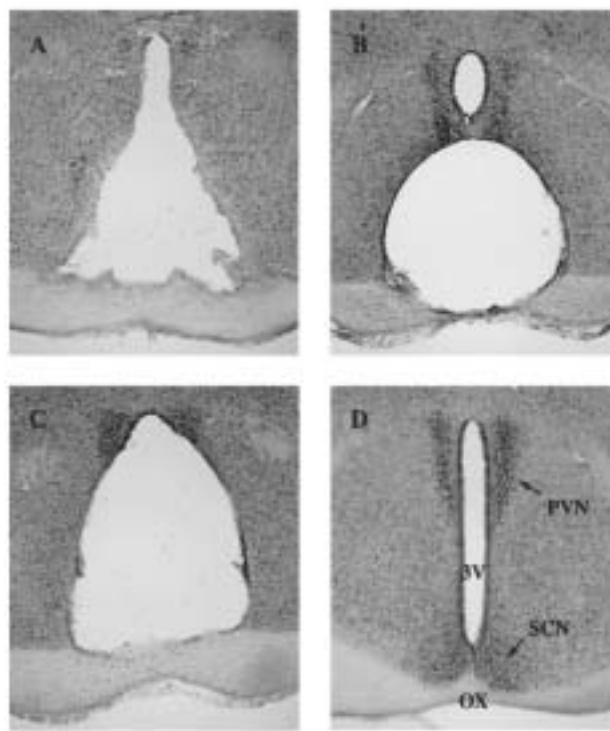


Figure 1. Representative photomicrographs ($\times 40$) of animals with complete ablation of the SCN (A-C) or sham surgery (D). PVN = paraventricular nucleus; SCN = suprachiasmatic nucleus; 3V = third ventricle; OX = optic chiasm.

Histology

One SCN_x hamster brain was lost during processing, but because of the complete lack of circadian rhythmicity in this animal's locomotor patterns based on visual inspection of the actograms and periodogram analysis, its data were included in the analysis. Seven of the 11 hamsters that failed to display rhythmic locomotor behavior after SCN_x sustained complete lesions of the SCN (Fig. 1). The remaining 3 hamsters had lesions that spared less than 10% of these nuclei and, because of the complete loss of rhythmic locomotor activity, were included in the analysis. Other structures damaged in the SCN_x group in addition to the optic chiasm were the following hypothalamic nuclei: periventricular nucleus, anterior hypothalamic area, medial preoptic nucleus, retrochiasmatic area, ventral paraventricular nucleus, and the ventromedial nucleus. These nuclei are listed in descending order of percent damage. In no animal did damage to any of these nuclei exceed 65%.

Six of the 9 hamsters that continued to express some component of circadian locomotor rhythmicity had lesions that spared 15% to 85% of the SCN. In addition, 5% to 10% of the SCN was spared in the remaining 3 hamsters, but because the actograms and periodograms showed evidence of circadian rhythmicity, these animals were not included in the analysis.

Preferred Hour

The preferred hour for torpor onset was established with the Rayleigh test (Fig. 2). Overall, 66.7% of the hamsters met the criteria for a preferred hour of torpor onset. A chi-square analysis determined that group percentages did not differ from the overall mean (AM-Sham, AM-SCNx, PM-Sham, and PM-SCNx = 83%, 40%, 86%, and 50%, respectively, $p > 0.05$). A second chi-square analysis that compared SCNx and Sham animals, collapsing across the time of feeding, indicated that a significantly lower percentage of SCNx hamsters displayed torpor onset at a preferred hour (45% vs. 85%, $p < 0.05$).

Hotelling's 1-sample test indicated that each group displayed torpor at a preferred hour ($p < 0.05$ in each case). Figure 3, which depicts the 95% confidence ellipses for each group, is a graphical representation of the results from the Hotelling's 1-sample test. None of the confidence ellipses encompasses the origin, indicating that these populations did not generate random onset vectors (Batschelet, 1981, pp 144-150). The center of each ellipse represents the center of gravity for that population, and the vector drawn from the origin to this point denotes the mean hour of torpor onset, also shown in Table 1.

Timing of Torpor Onset in Food-Restricted Hamsters

To minimize the chance of a Type I error due to the many statistical comparisons made, the significance level has been set at and presented as $p(\alpha) = 0.01$ in the 2 sections that follow. The results reported for all comparisons save 1 are, however, unchanged when $p(\alpha)$ is raised to 0.05; the mean clock time of torpor onset for the PM-SCNx group differs from that of AM-Sham animals when $p(\alpha) = 0.05$ (Table 1).

Photoperiod versus Feeding Schedule

AM- and PM-Sham groups were compared to determine the dominant zeitgeber in neurologically

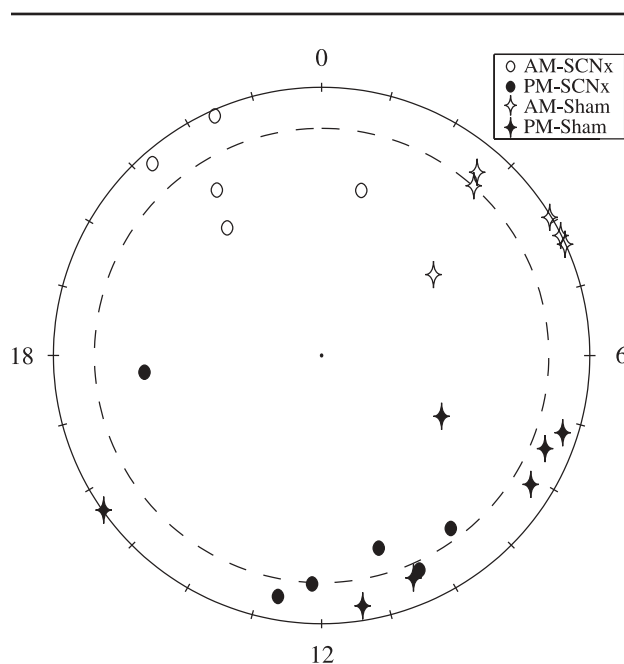


Figure 2. Rayleigh diagram of mean torpor onsets for individual hamsters plotted on a unit circle. Numbers outside the circle indicate clock time in hours. Each point represents the tip of the mean torpor onset vector for 1 individual hamster calculated by circular statistics. The dashed line demarcates the significance level of the Rayleigh test (radius = 0.847, $p(\alpha) = 0.05$). Hamsters whose points fall outside the dashed circle have a preferred hour for torpor onset. AM-SCNx and AM-Sham hamsters were provided with food in the morning, and PM-SCNx and PM-Sham animals were fed 12 h later in the evening.

Table 1. Mean Clock Time of Torpor Onset and Mean Latency from Food Presentation to Torpor Onset

	Clock Time (h:min)	Latency (h)
AM-Sham	3:38 ^a	20.64 ^a
AM-SCNx	22:16 ^b	15.26 ^b
PM-Sham	9:37 ^c	14.62 ^b
PM-SCNx	11:47 ^c	16.81 ^{a,b}

NOTE: Groups that do not contain the same superscript differ significantly from each other. Hotelling's 2-sample test, $p(\alpha) = 0.01$.

intact animals; AM-SCNx and PM-SCNx groups were compared to assess this relation in hamsters without a functional SCN circadian pacemaker. Torpor onsets in AM- and PM-Sham groups differed from each other in clock time ($p < 0.01$; Fig. 3A, Table 1), indicating that time of food presentation influenced the timing of torpor onset. The mean torpor onsets, however, differed by 6 h rather than the predicted 12-h difference expected if food presentation were the sole determining factor. When the onset of torpor relative to time of food presentation is considered, these groups again

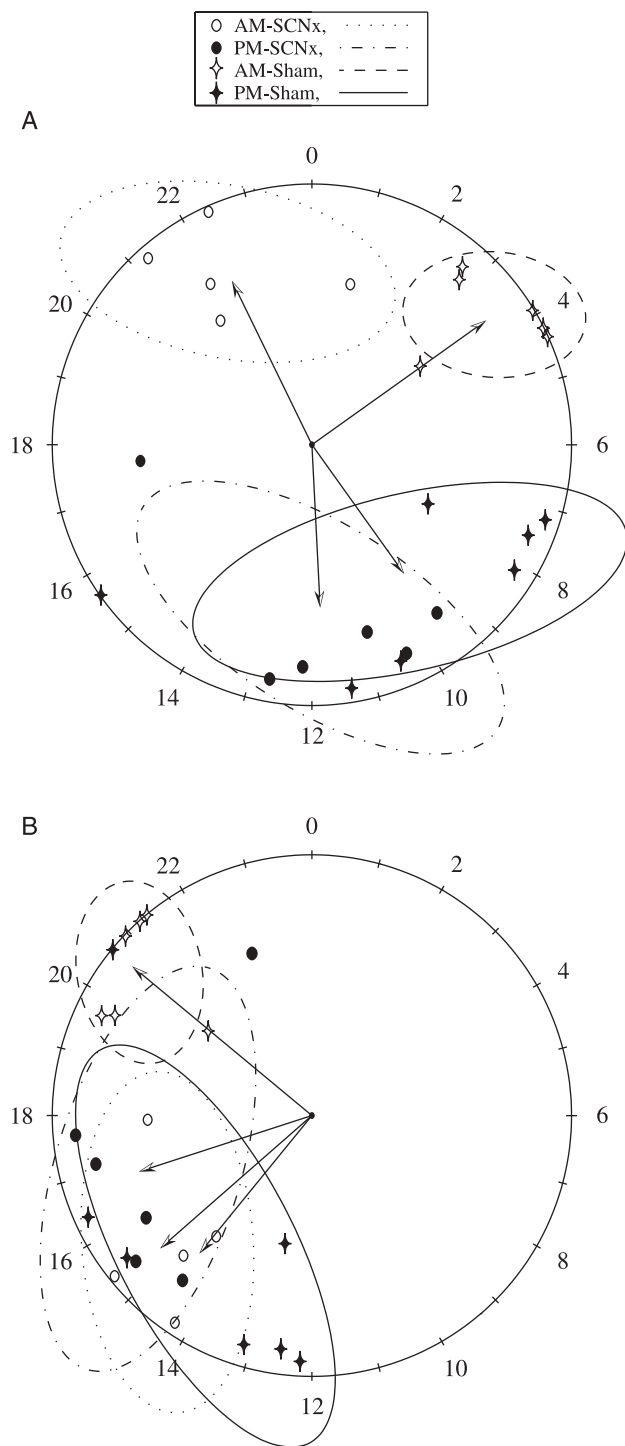


Figure 3. Hotelling's confidence ellipses and group mean vectors for torpor onset relative to clock time (A) and time of food presentation (B) plotted on a unit circle. Numbers outside the circle indicate the (A) clock time or (B) latency from food presentation in hours. Points represent mean torpor onsets for each hamster. Ellipses and vectors illustrate the 95% confidence interval and mean hour, respectively, for each group. All ellipses are clearly outside the origin, thereby indicating that each group has a significant preferred hour for torpor onset (Hotelling's 1-sample test, $p < 0.05$). Group designation as in Figure 2.

differ significantly ($p < 0.01$; Fig. 3B, Table 1), suggesting that food presentation is not the sole zeitgeber for timing of torpor.

The torpor onsets of AM-SCNx and PM-SCNx animals also differed in clock time but not relative to time of food presentation ($p < 0.01$ and $p > 0.05$, respectively; Fig. 3, Table 1). The mean clock times for torpor onset in these groups differ by approximately 10.5 h, which differs modestly from the predicted 12-h difference predicated on the timing of food presentation controlling the timing of torpor in animals lacking an SCN.

Importance of the SCN Circadian Pacemaker?

Food presentation evidently has a greater influence on the timing of torpor in hamsters that do not have a functional circadian pacemaker than in those with an intact SCN. To further assess the impact of SCN ablation on the timing of torpor onset, both SCNx groups were compared to their respective sham-ablated controls. PM-Sham and PM-SCNx torpor onsets did not differ in clock time or latency from food presentation ($p > 0.05$; Fig. 3, Table 1). AM-Sham and AM-SCNx torpor onsets did, however, differ relative to clock time and time of feeding ($p < 0.01$; Fig. 3, Table 1). SCN status apparently only affects the timing of torpor onset in the groups fed in the light phase (AM).

Other Torpor Characteristics

Representative torpor bouts for animals from each group are shown in Figure 4.

Torpor Duration

There was a significant overall effect for duration of torpor ($p < 0.05$), with a significant main effect of SCN status but not of feeding time ($p < 0.05$ and $p > 0.05$, respectively; Fig. 5A). Irrespective of feeding time, torpor bouts were shorter in SCNx than in sham-ablated hamsters ($p < 0.05$). The interaction between SCN status and feeding time was not significant ($p > 0.05$). Torpor bout duration was shorter in PM-SCNx than in PM-Sham hamsters ($p < 0.05$). The same overall pattern was evident in the AM-fed groups but fell short of statistical significance ($p = 0.08$).

$T_{b \min}$

The pattern of results for $T_{b \min}$ was identical to that for torpor duration (Fig. 5B). There was a main effect

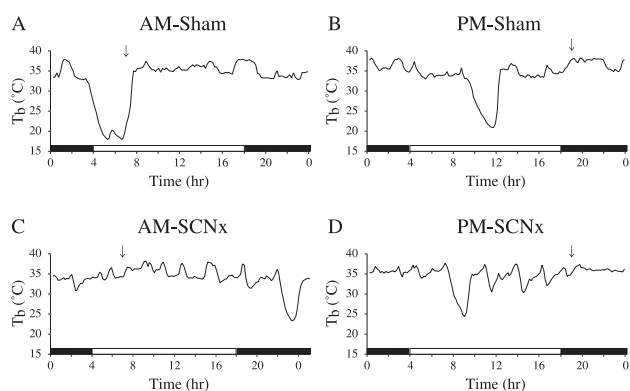


Figure 4. Representative torpor bouts of AM-Sham (A), PM-Sham (B), AM-SCNx (C), and PM-SCNx (D) hamsters. Vertical arrows indicate time of food presentation. The dark portion of the daily photocycle is denoted by the shaded bar on the abscissa. T_b = body temperature.

of SCN status ($p < 0.05$) but not of feeding time ($p > 0.05$). $T_{b \text{ min}}$ was higher in SCNx than in sham animals ($p < 0.05$). The interaction between SCN status and feeding time was not significant ($p > 0.05$). $T_{b \text{ min}}$ was significantly higher in PM-SCNx than in PM-Sham hamsters ($p < 0.05$) but not significantly so for the AM-SCNx versus AM-Sham comparison ($p = 0.10$). One animal in the PM-SCNx group was excluded from the above torpor duration and $T_{b \text{ min}}$ analyses because it differed from the group mean by more than 2 residual standard deviations in torpor duration (12.2 h) and $T_{b \text{ min}}$ (18.2 °C).

Body Mass

Animals did not differ in body mass prior to the experimental food restriction (31, 29, 35, and 31 g for AM-Sham, AM-SCNx, PM-Sham, and PM-SCNx groups, respectively, $p > 0.05$). Nor did hamster weights differ at the time of the first torpor bout (31, 27, 31, and 29 g for AM-Sham, AM-SCNx, PM-Sham, and PM-SCNx groups, respectively, $p > 0.05$). The percentage of baseline body mass at the time of the first torpor bout also did not differ between groups (100%, 96%, 90%, and 91% for AM-Sham, AM-SCNx, PM-Sham, and PM-SCNx groups, respectively, $p > 0.05$).

Locomotor Activity and FAA

During the interval of food restriction, 24-h activity counts were elevated above ad lib baseline levels for all but 2 hamsters. The mean increase was 112%. The

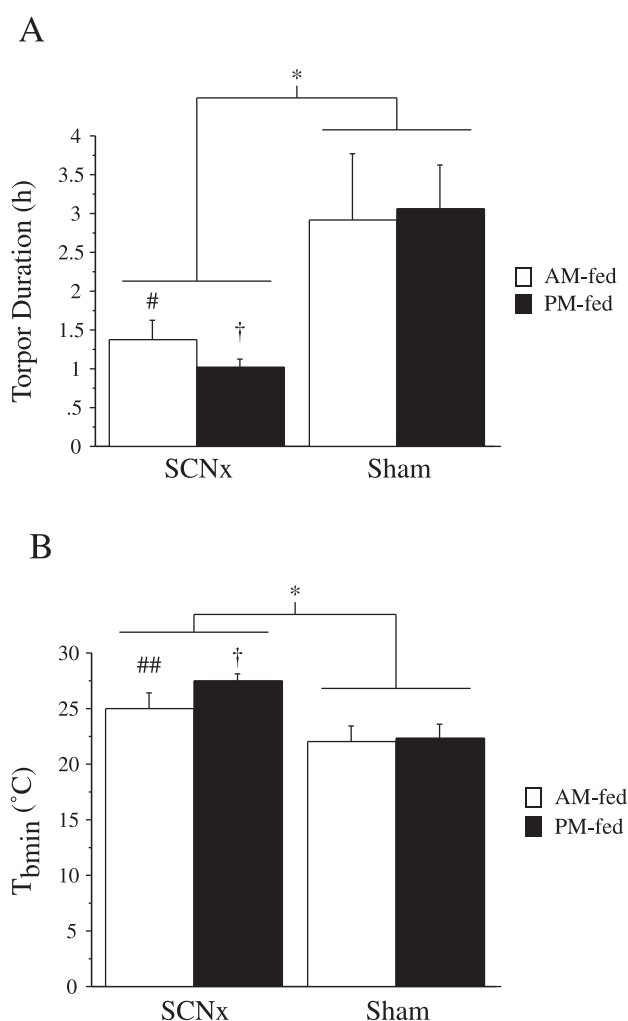


Figure 5. Mean torpor bout duration (A) and $T_{b \text{ min}}$ (B) for SCNx and Sham animals fed in the morning (0700 h; open bars) or evening (1900 h; shaded bars). * $p < 0.05$ SCNx versus Sham animals collapsed across time of feeding. † $p < 0.05$ compared to respective sham-ablated control. # $p = 0.08$ compared to sham-ablated control. ## $p = 0.10$ compared to sham-ablated control.

overall ANOVA did not reveal any differences among groups ($p > 0.05$).

Moderate to striking FAA was seen in 54% of hamsters. FAA occurred in animals from each group, with no group differences from the overall percentage (AM-Sham = 50%, AM-SCNx = 80%, PM-Sham = 57%, and PM-SCNx = 33%, $p > 0.05$). Figure 6A-D illustrates the best examples of FAA for each of the groups. Two animals, depicted in Figure 6E,F, failed to meet the statistical criterion for FAA, but visual inspection clearly indicated FAA. When the data from these animals are included in the analysis, 62.5% of hamsters displayed FAA; this inclusion does not change the outcome of the chi-square analysis ($p > 0.05$).

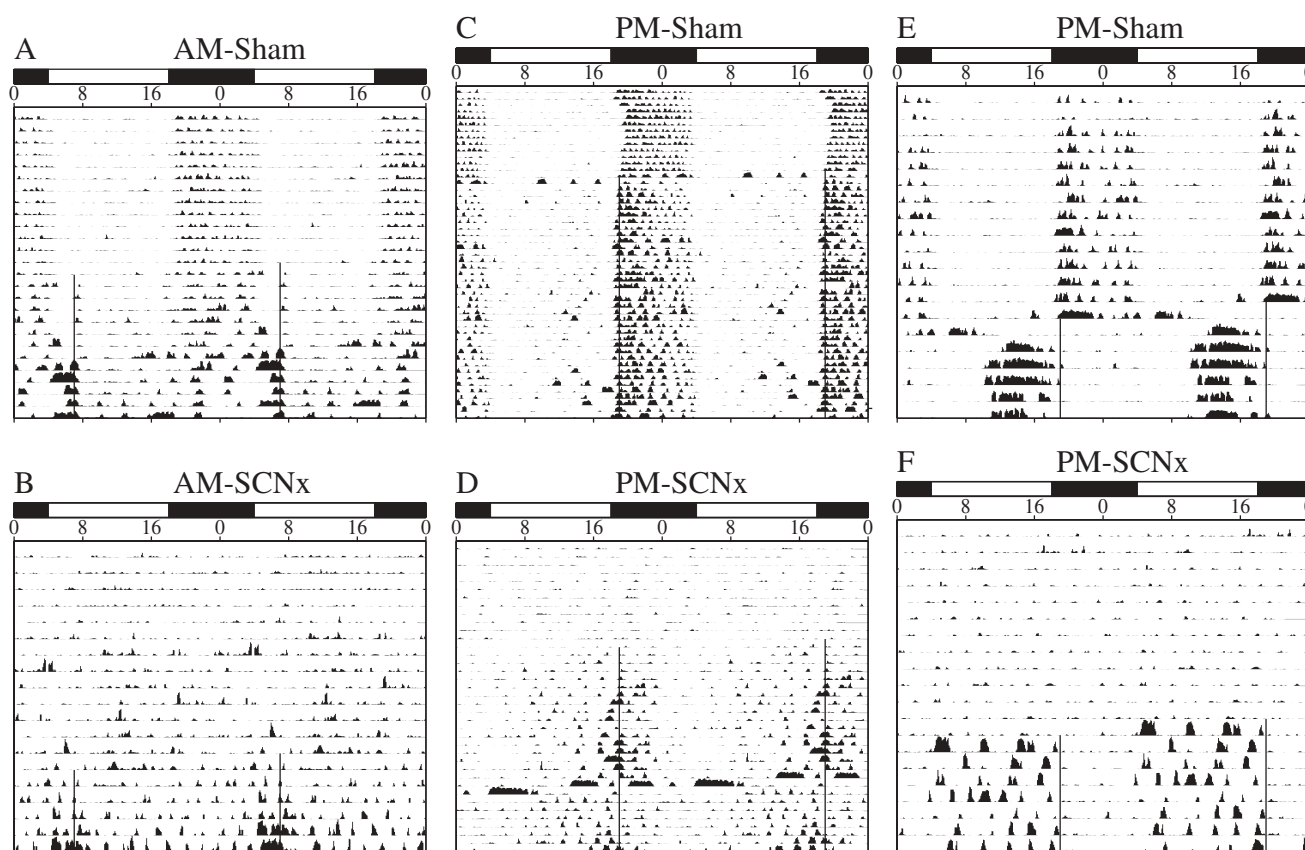


Figure 6. Double-plotted locomotor activity records of hamsters that displayed food anticipatory activity (FAA) based on statistical criteria (A, B, C, D; see Methods for details) or visual inspection (E, F). The dark portion of the daily photocycle is denoted by the shaded bar at the top of each actogram. Clock time is indicated below the light/dark cycle bar. Vertical lines indicate time of food presentation during the food restriction regimen. FAA was determined using data from the last 5 days of each actogram.

Qualitatively, SCN \times hamsters increased activity not only around the premeal interval (Fig. 6B,D,F); often, increased activity associated with feeding continued after food was presented (Fig. 6B,D). Activity increased throughout the light phase for most AM-fed sham animals but decreased and/or was disrupted at night (Fig. 6A). In the PM-Sham group, activity increased just prior to food presentation (Fig. 6C,E) and often ceased shortly after food presentation (Fig. 6E). In many of these animals, the pause in locomotor activity after FAA was brief or absent, and the nocturnal component of activity soon ensued (Fig. 6C).

Daily T_b Rhythm

Prior to and during food restriction, all sham-ablated hamsters displayed daily rhythms of T_b . The pattern of most AM-Sham hamsters during food restriction, however, was disrupted, reflecting suppression of elevated T_b during the dark phase. With the exception of 1 hamster that displayed a faint

rhythm, T_b rhythms were absent in all SCN \times animals prior to food restriction. During food restriction, periodicity of T_b emerged in all but 1 SCN \times hamster (data not shown).

DISCUSSION

Rhythmic Torpor and the Circadian Pacemaker

Individual and group analyses indicate that an intact SCN is not necessary for the expression of rhythmic torpor onset in food-restricted Siberian hamsters. Of the SCN \times hamsters that completely lacked circadian locomotor activity and T_b rhythms prior to food restriction, 45% displayed 24-h rhythms in torpor onset. With few exceptions, animals that failed to satisfy the statistical criterion for rhythmic torpor onset had mean onset vectors pointing to a similar clock time as other animals in their respective SCN \times group.

Hotelling's 1-sample test confirmed that all groups had a significant preferred torpor onset clock time.

These results appear to contradict earlier claims that an intact SCN is necessary for the expression of rhythmic torpor in food-restricted Siberian hamsters (Ruby and Zucker, 1992). The disparity can be reconciled by considering procedural differences. Ruby and Zucker (1992) food restricted their animals for relatively few days; once torpor was elicited, hamsters were fed *ad lib* thereafter. Many of the torpor bouts in their analyses occurred under *ad lib* feeding conditions and presumably while the animals were displaying arrhythmic feeding patterns (Cipolla-Neto et al., 1988). In the present experiment, food restriction was maintained throughout data collection, with food made available at a fixed clock time; under these conditions, hamsters consume much of their food in the first few hours after its presentation (Bae, 2000; Bae et al., 2003). According to our model, the SCN circadian pacemaker controls the timing of food intake, which in turn controls onset of torpor. Under the conditions of the Ruby and Zucker study, one would expect torpor to be arrhythmic while animals were fed *ad lib* and rhythmic under a fixed food restriction schedule.

Of the sham-ablated hamsters, 85% also had a preferred hour for torpor onset. This agrees with other reports of rhythmic torpor in intact Siberian hamsters (Ruf et al., 1989; Kirsch et al., 1991; Ruby and Zucker, 1992). A lower percentage of SCNx hamsters displayed rhythmic torpor onsets. This could be a consequence of the small number of torpor bouts (4) used to establish timing of torpor onsets, or it may reflect reduced rhythmicity in hamsters lacking normal circadian organization. The circadian output that governs torpor onset may not be under exclusive control by timing of food intake. Other neural or humoral SCN outputs not restored by rhythmic feeding behavior may contribute to timing of torpor.

Timing of Torpor Onset: Photoperiod, Food, and the Circadian System

AM- and PM-fed sham groups received their daily rations 12 h apart, but the difference in torpor onsets was 6 h, half of what is predicted by exclusive control of this behavior by photoperiod or time of feeding. In SCNx hamsters, torpor onsets largely overlapped for AM- and PM-fed hamsters when calculated as latencies from food presentation; by contrast, there was a 10.5-h difference in mean hour of torpor onset calcu-

lated relative to clock time. This suggests that both the photoperiod and the time of feeding affect the timing of torpor onset in intact hamsters. SCN ablation eliminates the effect of photoperiod, as would be expected by elimination of the retinohypothalamic tract that provides the major photic input to the circadian system (Morin, 1994). Nevertheless, it cannot be determined that this effect of photoperiod is independent of the behavioral feeding output pathway. Because food was not removed after a given interval from presentation, the differences in torpor onset in intact animals could reflect differences in feeding patterns not controlled for in this experiment. The nocturnal Siberian hamster consumes the majority of its food during the dark phase with a circadian suppression of food intake during the light (Ruf and Heldmaier, 1993). Thus, the AM-fed sham animals were the only group to receive food during an interval that opposed the normal circadian cycle in eating behavior. The intact circadian system of the AM-Sham animals may have caused these animals to postpone consumption of some of their daily food ration relative to other groups, thereby delaying torpor onset.

Hamsters do not fare well under food restriction, especially time-limited paradigms (Silverman and Zucker, 1976; Masuda and Oishi, 1995). To prevent serious health hazards associated with reduced food availability, hamsters must be restricted in the amounts of food made available each day rather than given access to unlimited food for just several hours each day. For this reason, food was not removed after a given interval from food presentation. Juvenile and adult Siberian hamsters allocated 75% of *ad lib* food rations 1 h before the dark phase consume 39% and 53%, respectively, of this meal within the first 45 min (Bae, 2000; Bae et al., 2003). In the present study, except in very few instances at the beginning of the food restriction regimen, all food had been consumed by the time of the next food presentation. We presume most of the food was ingested within the first few hours of presentation, but in the absence of direct measurement, we cannot rule out differences in feeding patterns between groups.

Time of food presentation unequivocally influenced the time of torpor onset in intact and SCNx Siberian hamsters. SCN control of the timing of torpor may occur at least in part via the same feeding-related pathway, as proposed by Le Minh et al. (2001) and Stokkan et al. (2001), for control of circadian oscillations in peripheral tissues. We propose that the free-running rhythms of torpor onset in constant darkness

(Ruf et al., 1989) and transients after shifts in the photocycle (Kirsch et al., 1991) are the result of parallel free-running rhythms and transients in feeding behavior.

The SCN and Torpor Characteristics

SCNx hamsters had shorter torpor bouts and higher $T_{b\ min}$ values during torpor than did sham-ablated controls, as reported previously by Ruby and Zucker (1992) for a subset of SCNx hamsters. This effect was more consistent in the present study; 9 of 11 SCNx hamsters had mean torpor durations < 1.5 h, and 8 of 11 had mean $T_{b\ min} > 26$ °C. In contrast, 11 of 13 sham-ablated hamsters had mean torpor durations > 1.5 h and $T_{b\ min} \leq 25$ °C. The consistent decrease in torpor duration and increase in $T_{b\ min}$ in SCNx hamsters could reflect extreme hyperprolactinemia common in SCNx Siberian hamsters (Bittman et al., 1991). Exogenous prolactin inhibits photoperiodically induced torpor (Ruby et al., 1993). Furthermore, hamsters given exogenous prolactin had higher $T_{b\ min}$ values during food-restricted torpor (25.3 ± 1.5 °C vs. 22.3 ± 1.1 °C), but this difference did not reach statistical significance (Ruby et al., 1993). Lactating Siberian hamsters also undergo shorter 2-deoxyglucose-induced torpor bouts than do nonlactating females (146 ± 17 min vs. 203 ± 33 min), but again this difference was not significant (Stamper et al., 1998). Elevated prolactin concentrations appear to predispose hamsters to shorter or more shallow torpor bouts. Alternatively, the decreased $T_{b\ min}$ and shortened torpor duration could be due to disruption of a timing mechanism that influences the time of arousal. SCNx hamsters did not appear to be less likely to display 4 torpor bouts, require more days of food restriction before torpor appeared, or sustain greater losses in body mass before they displayed torpor; SCNx affected only torpor duration and $T_{b\ min}$.

Food Anticipatory Activity

The incidence of FAA (63%) in the present study is similar to that reported for juvenile Siberian hamsters (69%) subject to food restriction (Bae et al., 2003). An intact SCN was not necessary for the expression of FAA, as previously reported for other rodents (Phillips and Mikulka, 1979; Stephan et al., 1979; Mistlberger, 1992; Marchant and Mistlberger, 1997). Furthermore, SCN damage did not affect the percentage of animals that manifested FAA. This differs from observations in Syrian hamsters in which FAA is sig-

nificantly more prevalent in SCNx than in intact hamsters (Abe and Rusak, 1992; Mistlberger, 1993). This may reflect species differences or procedural variations that included different food restriction and lighting regimens.

Model for Circadian Output and the Food-Entrained Oscillator (FEO)

On the basis of the persistence of food anticipatory rhythms in SCNx animals, an extra-SCN oscillator sensitive to feeding cues was postulated (Krieger et al., 1977; Stephan et al., 1979). The FEO was considered to interact with and normally be coupled to the light-entrained oscillator in the SCN.

The FEO has not been localized (reviewed in Stephan, 2002), possibly because multiple oscillators are widely distributed throughout the body. Rhythmic circadian clock gene expression throughout the brain (Abe et al., 2002) and body (Sakamoto et al., 1998) supports this conjecture. In common with FAA, many of these gene oscillations are sensitive to food restriction (Damiola et al., 2000; Stokkan et al., 2001; Wakamatsu et al., 2001) and persist in food-restricted SCNx (Hara et al., 2001) and *Clock* mutant (Minami et al., 2002; Oishi et al., 2002; Pitts et al., 2003) mice. It is possible that the several feeding-related rhythms, including FAA and torpor onset, are the result of food-induced shifts in core clock gene oscillations in extra-SCN tissues relevant to their circadian control. Thus, each of these tissues may be considered a separate FEO. Yet the link between extra-SCN clock gene expression and behavior/physiology remains to be demonstrated.

The feeding-related signal responsible for entrainment of rhythms remains unknown. Because food restriction affects rhythms throughout the body, a humoral cue is likely involved. This suspicion is strengthened by the findings that neither subdiaphragmatic vagotomy (Comperatore and Stephan, 1990) nor capsaicin-induced visceral deafferentation prevents FAA (Davidson and Stephan, 1998). Many studies have investigated several humoral signals related to feeding, but at present, it is not known which, if any, are important for feeding-related rhythms (for a review, see Stephan, 2002).

Food restriction has marked effects on behavioral, physiological, and gene circadian rhythms. How common or significant these changes are under normal conditions is unknown. Circadian fluctuations in food availability presumably occur naturally for relatively few species, predominantly predators (Zielinski,

1986), whereas food anticipatory rhythms occur in many species, including rabbits (Jilge et al., 1987; Jilge and Stahle, 1993), whose food availability does not fluctuate in a circadian fashion in nature. We propose that it is the circadian pacemaker's control over rhythms in eating behavior, rather than fluctuations in food availability, that imparts timing information. In this view, food entrainment is not a rare phenomenon that occurs only when food availability is restricted to 1 phase of the diurnal cycle; rather, it occurs daily under ad lib feeding conditions, where nocturnal animals consume substantially more food during the subjective night than day.

Conclusions

Food restriction maintains diurnal rhythmicity of daily torpor onset in a subset of SCN \times Siberian hamsters and influences the circadian timing of torpor onset in both SCN \times and intact animals. Thus, onset of torpor may be added to the list of circadian rhythms affected by food restriction. This is consistent with the proposal that the SCN imparts circadian timing information for torpor onset through its control of feeding behavior.

ACKNOWLEDGMENTS

We are grateful to Maryam Shahdi, Julia Moritis, Chris Tuthill, Kim Pelz, and Matthew Butler for excellent technical and statistical assistance. Matthew Butler, John Dark, and Jin Ho Park provided helpful criticism of the manuscript. This research was supported by grant MH-61171 from the National Institute of Mental Health.

REFERENCES

- Abe H and Rusak B (1992) Anticipatory activity and entrainment of circadian rhythms in Syrian hamsters exposed to restricted palatable diets. *Am J Physiol* 263:R116-R124.
- Abe M, Herzog ED, Yamazaki S, Straume M, Tei H, Sakaki Y, Menaker M, and Block GD (2002) Circadian rhythms in isolated brain regions. *J Neurosci* 22:350-356.
- Allen G, Rappé J, Earnest DJ, and Cassone VM (2001) Oscillating on borrowed time: Diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts. *J Neurosci* 21:7937-7943.
- Bae HH (2000) Development and temporal timing of torpor in Siberian hamsters (*Phodopus sungorus*). PhD dissertation, University of California, Berkeley.
- Bae HH, Larkin JE, and Zucker I (2003) Juvenile Siberian hamsters display torpor and modified locomotor activity and body temperature rhythms in response to reduced food availability. *Physiol Biochem Zool* 76: 858-867.
- Batschelet E (1981) *Circular Statistics in Biology*. Academic Press, London.
- Bittman EL, Bartness TJ, Goldman BD, and DeVries GJ (1991) Suprachiasmatic and paraventricular control of photoperiodism in Siberian hamsters. *Am J Physiol* 260:R90-R101.
- Boulos Z, Rosenwasser AM, and Terman M (1980) Feeding schedules and the circadian organization of behavior in the rat. *Behav Brain Res* 1:39-65.
- Cipolla-Neto J, Afeche SC, Menna-Barreto L, Marques N, Benedito-Silva AA, Fortunato G, Recine EG, and Schott C (1988) Lack of similarity between the effect of lesions of the suprachiasmatic nucleus and subparaventricular hypothalamic zone on behavioral circadian rhythms. *Braz J Med Biol Res* 21:653-654.
- Comperatore CA and Stephan FK (1990) Effects of vagotomy on entrainment of activity rhythms to food access. *Physiol Behav* 47:671-678.
- Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, and Schibler U (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* 14:2950-2961.
- Davidson AJ and Stephan FK (1998) Circadian food anticipation persists in capsaicin deafferented rats. *J Biol Rhythms* 13:422-429.
- Elliott JA, Bartness TJ, and Goldman BD (1987) Role of short photoperiod and cold exposure in regulating daily torpor in Djungarian hamsters. *J Comp Physiol [A]* 161:245-253.
- Geiser F (1988) Reduction of metabolism during hibernation and daily torpor in mammals and birds: Temperature effect or physiological inhibition? *J Comp Physiol [B]* 158:25-37.
- Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, and Shibata S (2001) Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells* 6:269-278.
- Heldmaier G and Ruf T (1992) Body temperature and metabolic rate during natural hypothermia in endotherms. *J Comp Physiol [B]* 162:696-706.
- Heldmaier G and Steinlechner S (1981) Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus*. *Oecologia* 48:265-270.
- Hill RW (1975) Daily torpor in *Peromyscus leucopus* on an adequate diet. *Comp Biochem Physiol A* 51:413-423.
- Hudson JW (1978) Shallow daily torpor: A thermoregulatory adaptation. In *Strategies in the Cold*, JW Hudson, LCH Wang, eds, pp 67-108, Academic Press, New York.
- Jilge B, Hornicke H, and Stahle H (1987) Circadian rhythms of rabbits during restrictive feeding. *Am J Physiol* 253:R46-R54.
- Jilge B and Stahle H (1993) Restricted food access and light-dark: Impact of conflicting zeitgebers on circadian rhythms of the rabbit. *Am J Physiol* 264:R708-R715.
- Kirsch R, Ouarour A, and Pevet P (1991) Daily torpor in the Djungarian hamster (*Phodopus sungorus*): Photoperiodic

- regulation, characteristics and circadian organization. *J Comp Physiol [A]* 168:121-128.
- Krieger DT, Hauser H, and Krey LC (1977) Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. *Science* 197:398-399.
- Le Minh N, Damiola F, Tronche F, Schutz G, and Schibler U (2001) Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *EMBO J* 20:7128-7136.
- Lynch GR, Sullivan JK, and Gendler SL (1980) Temperature regulation in the mouse, *Peromyscus leucopus*: Effects of various photoperiods, pinealectomy and melatonin administration. *Int J Biometeorol* 24:49-55.
- Marchant EG and Mistlberger RE (1997) Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6j mice. *Brain Res* 765:273-282.
- Masuda A and Oishi T (1995) Effects of restricted feeding on the light-induced body weight change and locomotor activity in the Djungarian hamster. *Physiol Behav* 58:153-159.
- Minami Y, Horikawa K, Akiyama M, and Shibata S (2002) Restricted feeding induces daily expression of clock genes and *Pai-1* mRNA in the heart of *Clock* mutant mice. *FEBS Lett* 526:115-118.
- Mistlberger RE (1992) Nonphotic entrainment of circadian activity rhythms in suprachiasmatic nuclei-ablated hamsters. *Behav Neurosci* 106:192-202.
- Mistlberger RE (1993) Circadian properties of anticipatory activity to restricted water access in suprachiasmatic-ablated hamsters. *Am J Physiol* 264:R22-R29.
- Morhardt JE and Hudson JW (1966) Daily torpor induced in white-footed mice (*Peromyscus* spp.) by starvation. *Nature* 212:1046-1047.
- Morin LP (1994) The circadian visual system. *Brain Res Rev* 19:102-127.
- Oishi K, Miyazaki K, and Ishida N (2002) Functional CLOCK is not involved in the entrainment of peripheral clocks to the restricted feeding: Entrainable expression of *mPer2* and *BMAL1* mRNAs in the heart of *Clock* mutant mice on Jcl:ICR background. *Biochem Biophys Res Commun* 298:198-202.
- Phillips JL and Mikulka PJ (1979) The effects of restricted food access upon locomotor activity in rats with suprachiasmatic nucleus lesions. *Physiol Behav* 23:257-262.
- Pitts S, Perone E, and Silver R (2003) Food-entrained circadian rhythms are sustained in arrhythmic *Clk/Clk* mutant mice. *Am J Physiol* 285:R57-R67.
- Ruby NF, Ibuka N, Barnes BM, and Zucker I (1989) Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *Am J Physiol* 257:R210-R215.
- Ruby NF, Nelson RJ, Licht P, and Zucker I (1993) Prolactin and testosterone inhibit torpor in Siberian hamsters. *Am J Physiol* 264:R123-R128.
- Ruby NF and Zucker I (1992) Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *Am J Physiol* 263:R353-R362.
- Ruf T and Heldmaier G (1992) The impact of daily torpor on energy requirements in the Djungarian hamster, *Phodopus sungorus*. *Physiol Zool* 65:994-1010.
- Ruf T and Heldmaier G (1993) Individual energetic strategies in winter-adapted Djungarian hamsters: The relation between daily torpor, locomotion, and food consumption. In *Life in the Cold*, C Carey, GL Florant, BA Wunder, B Horowitz, eds, pp 99-107, Westview, Boulder, CO.
- Ruf T, Steinlechner S, and Heldmaier G (1989) Rhythmicity of body temperature and torpor in the Djungarian hamster, *Phodopus sungorus*. In *Living in the Cold II*, A Malan, B Canguilhem, eds, pp 53-61, Libbey Eurotext, London.
- Sakamoto K, Nagase T, Fukui H, Horikawa K, Okada T, Tanaka H, Sato K, Miyake Y, Ohara O, Kako K, et al. (1998) Multitissue circadian expression of rat period homolog (*rPer2*) mRNA is governed by the mammalian circadian clock, the suprachiasmatic nucleus in the brain. *J Biol Chem* 273:27039-27042.
- Silverman HJ and Zucker I (1976) Absence of post-fast food compensation in the golden hamster (*Mesocricetus auratus*). *Physiol Behav* 17:271-285.
- Stamper JL, Zucker I, Lewis DA, and Dark J (1998) Torpor in lactating Siberian hamsters subjected to glucoprivation. *Am J Physiol* 274:R46-R51.
- Stephan FK (1981) Limits of entrainment to periodic feeding in rats with suprachiasmatic lesions. *J Comp Physiol [A]* 143:401-410.
- Stephan FK (1984) Phase shifts of circadian rhythms in activity entrained to food access. *Physiol Behav* 32:663-671.
- Stephan FK (1986) The role of period and phase in interactions between feeding- and light-entrainable circadian rhythms. *Physiol Behav* 36:151-158.
- Stephan FK (2002) The "other" circadian system: Food as a zeitgeber. *J Biol Rhythms* 17:284-292.
- Stephan FK, Swann JM, and Sisk CL (1979) Anticipation of 24-hr feeding schedules in rats with lesions of the suprachiasmatic nucleus. *Behav Neural Biol* 25:346-363.
- Stephens MA (1969) Tests for randomness of directions against two circular alternatives. *Am Stat Assoc J* 64:280-289.
- Stokkan KA, Yamazaki S, Tei H, Sakaki Y, and Menaker M (2001) Entrainment of the circadian clock in the liver by feeding. *Science* 291:490-493.
- Strubbe JH, Prins AJ, Bruggink J, and Steffens AB (1987) Daily variation of food-induced changes in blood glucose and insulin in the rat and the control by the suprachiasmatic nucleus and the vagus nerve. *J Auton Nerv Syst* 20:113-119.
- Wakamatsu H, Yoshinobu Y, Aida R, Moriya T, Akiyama M, and Shibata S (2001) Restricted-feeding-induced anticipatory activity rhythm is associated with a phase-shift of the expression of *mPer1* and *mPer2* mRNA in the cerebral cortex and hippocampus but not in the suprachiasmatic nucleus of mice. *Eur J Neurosci* 13:1190-1196.
- Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, and Tei H (2000) Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288:682-685.
- Zielinski WJ (1986) Circadian rhythms of small carnivores and the effect of restricted feeding on daily activity. *Physiol Behav* 38:613-620.