

Nonphotic Entrainment of Circadian Activity Rhythms in Suprachiasmatic Nuclei–Ablated Hamsters

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This study examined the role of the suprachiasmatic nuclei (SCN) in nonphotic entrainment. The wheel-running activity of SCN-ablated hamsters was recorded in constant dark (DD) and then under prolonged schedules of 2-hr daily cage changes, restricted food availability, and daily light–dark (LD) cycles. Some hamsters with very large lesions subsuming the SCN and surrounding areas exhibited significant, albeit unstable, circadian activity rhythms in DD. Some hamsters with similar ablations also showed entrained rhythms to daily cage change schedules. All hamsters showed robust rhythms entrained to a daily feeding schedule, but no hamsters showed entrainment to LD cycles. Competent circadian oscillators evidently exist outside the SCN, at least 0.5 mm or more away, and at least some are nonphotically entrainable. Weaker entrainment in animals with larger lesions suggests that nonphotically entrainable oscillators also exist within the SCN or its immediate vicinity.

In the hamster, ablation of the hypothalamic suprachiasmatic nuclei (SCN) eliminates or severely disrupts circadian rhythms in constant environmental conditions and their entrainment by daily light–dark (LD) cycles (Moore & Eichler, 1972; Rusak, 1977; Stephan & Zucker, 1972; Stetson & Watson-Whitmyre, 1976). Convergent evidence from recording (Green & Gillette, 1982; Inouye & Kawamura, 1979), stimulation (Rusak & Groos, 1982), and transplant studies (Ralph, Foster, Davis, & Menaker, 1990) indicates that the rodent SCN function as a primary circadian pacemaker that mediates photic entrainment via a direct input from the retina (Johnson, Moore, & Morin, 1988; Moore & Lenn, 1972). Other observations suggest two additional levels of complexity within the rodent circadian system. First, competent circadian oscillators appear to exist outside of the SCN in hamsters (Rusak, 1977) and rats (Stephan, Swann, & Sisk, 1979). Second, circadian rhythms in hamsters and other rodents can be entrained by nonphotic stimuli, such as daily schedules of cage changes and social interactions (Mrosovsky, 1988), forced treadmill running (Mistlberger, 1991), restricted foraging (Rusak, Mistlberger, Losier, & Jones, 1989), or restricted food availability (Abe & Ebihara, 1990; Stephan et al., 1990). Neural mechanisms mediating nonphotic entrainment are unknown. The present study was designed to determine whether the SCN are necessary for nonphotic entrainment in the hamster by exposing SCN-ablated hamsters to a sequence of cage change and restricted feeding schedules that were separated by extended recordings under constant, undisturbed conditions and, in some cases, also under LD cycles. Large and small total SCN

ablations were used in an effort to determine whether nonphotically entrainable oscillators are distributed within, around, or at a distance from the SCN, as defined by Nissl staining.

Method

Two groups of adult male hamsters (LVG/LAK:SY) were obtained from Charles River (Montreal, Quebec, Canada) at 8 weeks of age and group housed in a colony room with controlled lighting (LD cycle = 12:12 hr) and temperature (21 °C) for 2 weeks. Animals in Group 1 ($n = 9$) were part of a study of food hoarding behavior. These animals were recorded in individual plastic wheel-running cages (45 × 25 × 20 cm) under the 12:12-hr LD cycle (LD 12:12) for 3 weeks during which they were provided access to a foraging area for 20 min every other day to collect food (sunflower seeds and Purina rat chow pellets). Food supplements were provided as necessary to maintain body weights above 85% of their starting weight. The animals were then subjected to bilateral radio frequency lesions (15 mA, 15 s, Grass LM3 lesion maker, Quincy, MA) of the SCN produced by a stereotactically guided “000” insect pin that was insulated to within 0.5 mm of its pointed tip. Stereotaxic coordinates were 2.9 mm anterior to bregma, 7.8 mm ventral to the dura, and ± 0.3 mm lateral to the midline (mouth piece at 5 mm). Wheel-running activity was then recorded for another 24 days after which a 15-day restricted foraging schedule was repeated. Lighting was continuous dim red (LLdim; 1 lx).

For the next 30 days, food and water were provided freely and the hamsters were subjected to a daily 2-hr cage change beginning at the former foraging time. During the cage changes, the hamsters were manually transferred into an empty neighboring cage. If an animal became quiescent during the 2-hr test, then it was gently prodded. After the last cage change day, a final 20 days of wheel running were recorded. The animals were then overdosed with pentobarbital and perfused intracardially with saline and formalin. The brains were removed and stored in fixative until they were sectioned on a cryostat (set for a thickness of 40 μ m) and mounted for cresyl violet Nissl staining.

The Group 2 hamsters ($n = 18$) were first subjected to radio frequency SCN ablation using four lesion placements instead of two. The coordinates were 2.8 and 3.1 mm anterior to bregma, 7.8 mm

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ventral to the dura, and ± 0.3 mm lateral to the midline. The animals were then housed in individual plastic wheel-running cages in LLdim. After 30 days of recording with minimal disturbance, a daily cage change schedule was implemented for 30 days. Each hamster was removed from its cage and locked in the wheel of a Wahmann (Baltimore, MD) wheel-running cage for 2 hr. This schedule was followed by 11 days of undisturbed recording. LD 12:12 was then implemented for 11 days, which was followed by another 13 days of undisturbed recording. A food and water restriction schedule was then enforced for 30 days during which the hamsters were provided with an apple slice, sunflower seeds, and a chow pellet every 23.83 hr (i.e., 10 min earlier each day). Water was available for 2 hr beginning at that time. The animals were weighed, and food amounts were adjusted daily to ensure that body weights did not drop below 85% of the starting weight. The animals were then recorded for 14 days under free-feeding and free-drinking conditions before being sacrificed and perfused for histological analysis.

Wheel running was recorded by microswitch closures that were monitored continuously by Apple II computers. Activity counts were summed and stored at 10-min intervals and periodically downloaded to a Macintosh FX computer for incorporation into individual data files.

Data were analyzed using the Circadia software package (Behavioral Cybernetics, Cambridge, MA). Activity rhythms were assessed visually in the form of actograms and average waveforms and quantitatively by periodogram analysis (after Dorrscheidt & Beck, 1975).

Results

Histology

Schematics of representative lesions are provided in Figure 1. Lesions in Group 1 animals were small and confined to the SCN area. Seven of 9 animals were judged to have sustained complete SCN ablation (e.g., Figures 1C–1E). Two animals sustained partial ablations that spared 20–50% of the SCN ventrolaterally. Minor damage to the periventricular nuclei (Pe) dorsal to the SCN and to the optic chiasm ventral to the SCN was also evident in all animals.

Lesions in Group 2 animals were, as intended, generally much larger. The SCN were judged as unambiguously destroyed in 15 of 18 animals (e.g., Figures 1F–1I) and probably

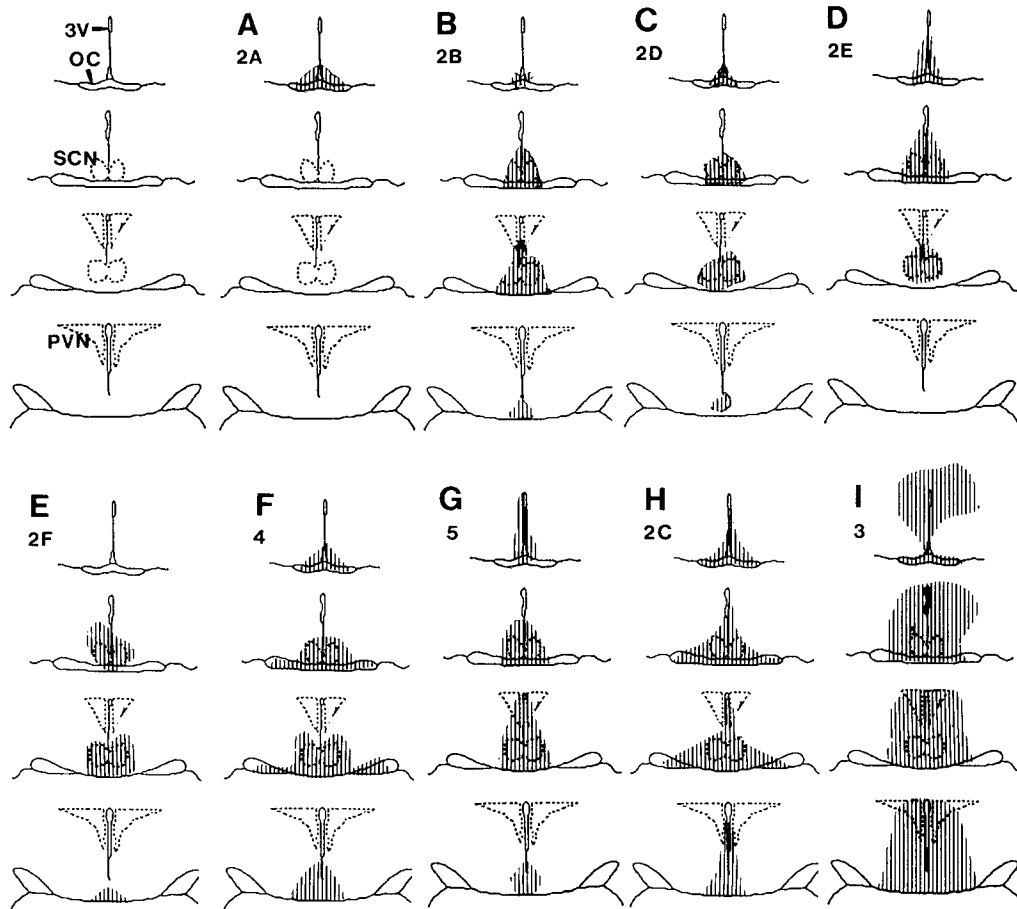


Figure 1. Schematics of coronal sections at four levels through the hamster hypothalamus, just anterior to the suprachiasmatic nuclei (SCN), medial-anterior and medial-posterior SCN, and just posterior to the SCN. (Stippling indicates lesion cavity and zone of complete gliosis. The figures are arranged in order [A–I] from the smallest to the largest lesions. Numbers refer to corresponding activity chart figure. OC = optic chiasm; PVN = paraventricular nuclei.)

destroyed in 1 other animal (Figure 1B, small lesion cavity but no detectable SCN). Two animals sustained partial SCN destruction (e.g., Figure 1A). Additional structures damaged in most animals included the hypothalamic paraventricular nuclei (PVN, ventral and medial), medial preoptic area (MPOA), preoptic pars suprachiasmatic area, anterior hypothalamic nuclei (AH, medial), Pe (usually extensive), arcuate nuclei (Arc), and optic chiasms.

Activity Rhythms

Activity charts from 9 ablated animals are presented in Figures 2–5. These animals illustrate the range of effects observed under the various conditions of the study (see Figure 1 for matching histology).

LLdim. Animals with partial lesions of the SCN ($n = 4$) exhibited apparently normal free-running activity rhythms in LLdim, with stable periods (τ) that ranged from 24–24.16 hr (e.g., Figure 2A). Animals with no detectable SCN tissue ($n = 23$) exhibited abnormal or severely disrupted activity rhythms. One of these animals (Figure 2B) displayed a unimodal circadian activity component that was characterized by a short, unstable τ (23.5–24.0 hr) and an unstable phase. This animal's lesion (Figure 1B) was restricted to the SCN area and damaged little else other than ventral Pe and subjacent chiasm. Two other animals (Figure 2C, Sx6; Figure 3, Sx13) displayed multiple rhythmic activity components that alternately appeared to independently run free or interact. These components were unstable in phase and period. Both animals had sustained unambiguously complete SCN ablation. Animal Sx6 also sustained minor damage to the PVN and MPOA and extensive damage to the Pe and retrochiasmatic–Arc areas (Figure 1H). Animal Sx13 sustained the largest lesion of all, with near complete bilateral destruction of the magnocellular PVN and Pe and extensive damage to the MPOA and the retrochiasmatic–Arc areas (Figure 1I).

Three animals displayed very low levels of activity after surgery; in each case wheel running was essentially absent after 2–4 weeks. No circadian organization was detected before this. Lesions in these animals tended to have an anterior focus and damaged the MPOA and rostral perichiasmatic areas, with relatively minor damage to the retrochiasmatic areas.

The remaining 17 animals with complete SCN ablations exhibited stable levels of activity with no obvious circadian organization (Figures 2D–2F, 4, and 5). Their activity records appeared arrhythmic by visual inspection, although periodogram analysis in some cases detected rhythms in the 12–18-hr or 20–30-hr ranges that were barely significant at the 95% confidence level (e.g., Figures 4B and 5B). Similar marginally significant periodicities were also occasionally detected in random number data sets. Lesions in these animals (e.g., Figures 1C–1G, respectively) ranged in size from small to large but were similar in focus to animals Sx6 and Sx13.

Cage change schedule. All hamsters immediately ran in the wheels during the daily 2-hr cage change. Gentle prodding was occasionally necessary to maintain this. Three types of circadian effects were observed. Animals with partial SCN ablations exhibited entrainment of free-running rhythms to the daily cage change schedule (e.g., Figure 2A). In each case, the

10–12-hr active phase of the entrained rhythm began immediately after the daily cage change session. When the schedule was terminated, the activity rhythm free-ran from the apparent phase of entrainment. On the 1st day of free-run, activity onset began 0.5–1.5 hr after the usual time of cage change.

Animals with apparently complete SCN ablation exhibited very different effects. Seven animals showed no obvious synchrony to the cage change sessions. In three cases, average waveforms of the last 20 days of the schedule revealed a tendency for more activity in the 6–10 hr that preceded the daily scheduled cage change. In the other four cases, the average waveforms were virtually flat. These latter cases included animals Sx6 (Figure 2C) and Sx13 (Figure 3), both of which exhibited significant, albeit unstable, circadian components during LLdim baseline recording. None of these 7 hamsters showed any evidence of circadian organization under LLdim after the cage change schedule. Lesions in these animals were moderate to large in size, and complete SCN ablation was unambiguous.

The remaining animals with complete SCN ablation did show evidence of synchrony to the cage change schedule. Synchrony was in each case manifested as a small band of activity that preceded the daily cage change sessions by 6–10 hr (e.g., Figures 2B, 2D–2F, 4, and 5). In some cases, this activity bout was very weak and unstable (Figure 4). A second activity bout sometimes occurred immediately before the cage change sessions (e.g., Figures 2D, 2F, and 5), but this was usually less distinct. When the schedule was terminated, 6 animals showed a free-running, sometimes bimodal, activity rhythm with a short, unstable τ (e.g., Figures 2B, 2E, and 2F). This pattern was unstable; in two cases it became trimodal (e.g., Figures 2B and 2F), whereas in the other cases it decomposed further and became apparently random. These animals tended to have smaller lesions that were confined to the immediate SCN area. Other animals that exhibited similar synchrony to the cage change schedules showed either immediate or rapid (within 2–3 days) decomposition to a disorganized or apparently random activity pattern when the schedule was terminated; that is, there was little or no evidence for a free-running rhythm in LLdim (e.g., Figures 2D, 4, and 5). Most of these latter animals had moderate-to-large lesions with unambiguous destruction of the SCN.

LD 12:12. Twelve animals in Group 2 were recorded under LD 12:12 for up to 11 days (2 animals were sacrificed after 8 and 10 days, respectively; Figures 2B and 2C). Two animals showed no synchrony to LD 12:12. Both animals showed extensive damage to the optic chiasm. The remaining 10 animals showed predominantly nocturnal activity under LD 12:12. In some cases, activity was virtually absent during the lights-on period (e.g., Figure 3) but was at normal levels during the dark period. In other cases, nocturnality was less evident in the actograms but was clearly revealed in average waveforms (e.g., Figures 4 and 5). All of these animals sustained some damage to the optic chiasm that ranged from minor to extensive. After return to LLdim, none of the animals tested ($n = 8$) exhibited evidence of a free run; 24-hr activity rhythms were completely absent even on Day 1 of LLdim. Periodograms in some animals occasionally detected significant

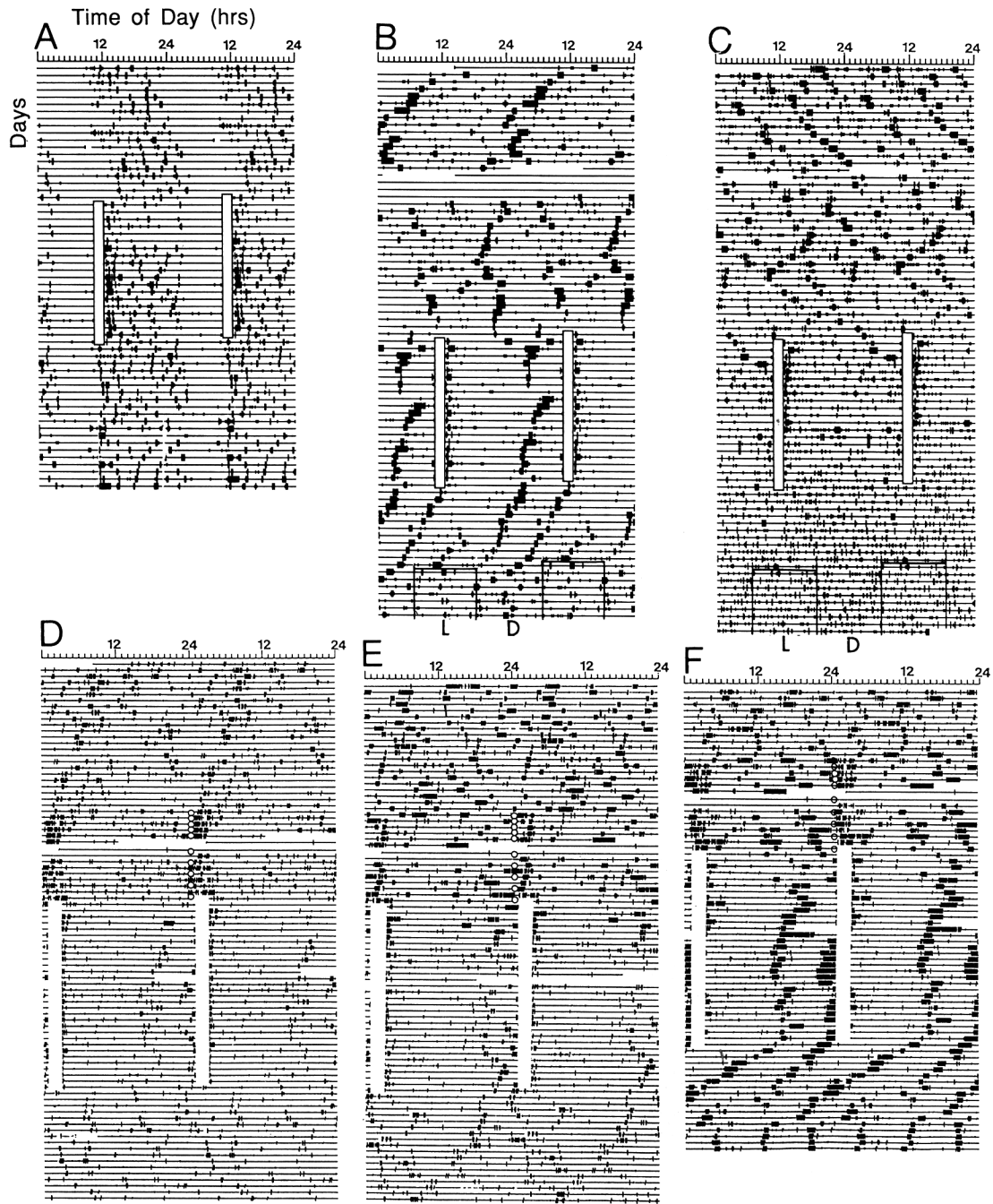


Figure 2. Double-plotted actograms from 6 suprachiasmatic nuclei-ablated hamsters (A–F) subjected to a 2-hr cage change schedule in constant dim red light. (Each line represents 48 hr, with time plotted from left to right in consecutive 10-min bins. Vertical deflections are proportional to amount of activity in the bin. Flatline indicates no activity in that bin. Animals D–F were permitted to explore an open field to collect food for 30 min at the times indicated by the open circles. Blank sections indicate the times of daily cage changes [other blanks reflect missing data]. Animals A–C were also recorded under a 12:12-hr light–dark cycle as indicated by the solid lines.)

periodicities in the 20–30-hr range, but these were isolated and marginal at the .05 level.

Food restriction. Eight animals in Group 2 were recorded under a food restriction schedule. The responses were uniform

and dramatic; all animals showed emergence of a robust daily rhythm of wheel running with a stable, positive (anticipatory) phase in relation to feeding time. Average waveforms plotted at 23.83 hr (period of the feeding cycle) revealed that maximal

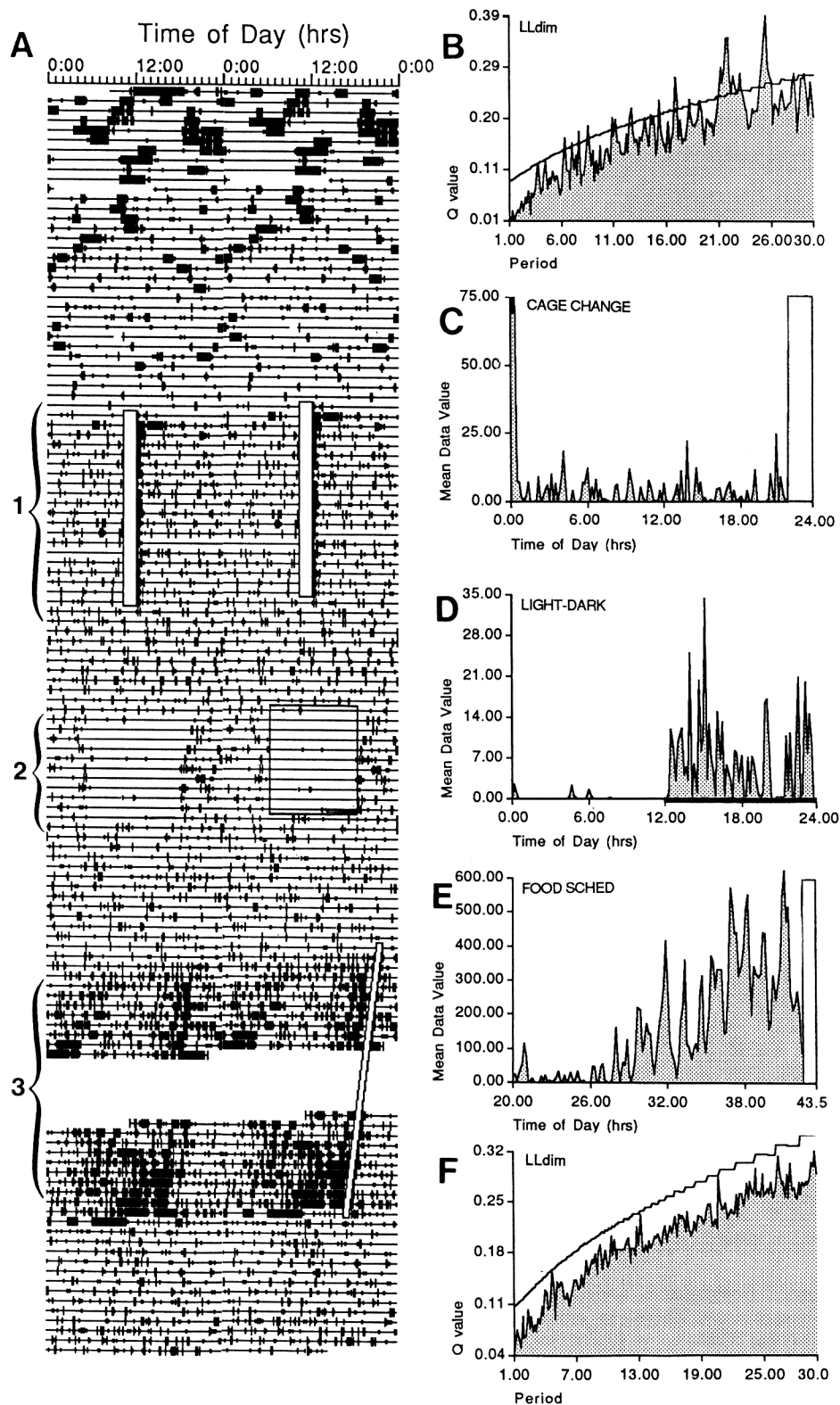


Figure 3. Activity charts for a hamster (animal Sx13) with a very large, complete suprachiasmatic nuclei ablation. (A: Double-plotted actogram recorded in dim light [LLdim], during a daily cage change schedule [Bracket 1], a 12:12-hr light–dark cycle [Bracket 2; solid lines block out the lights-on period], and a daily food restriction schedule [Bracket 3]. B: Periodogram analysis of the last 20 days of constant LLdim before cage change schedule; the stepping line represents the 95% confidence interval [*F* distribution]. C: Average waveform of the last 20 days of cage change schedule; the 2-hr cage change period is indicated by the open vertical bar. D: Average waveform of 11 days of the 12:12-hr light–dark cycle; lights-off period is indicated by the filled horizontal bar. E: Educated waveform [plotted at 23.83 hr] of the last 10 days of restricted feeding and drinking; the first 30 min of food availability is indicated by the open vertical bar. F: Periodogram analysis of the first 12 days of ad-lib food and water after the restriction schedule.)

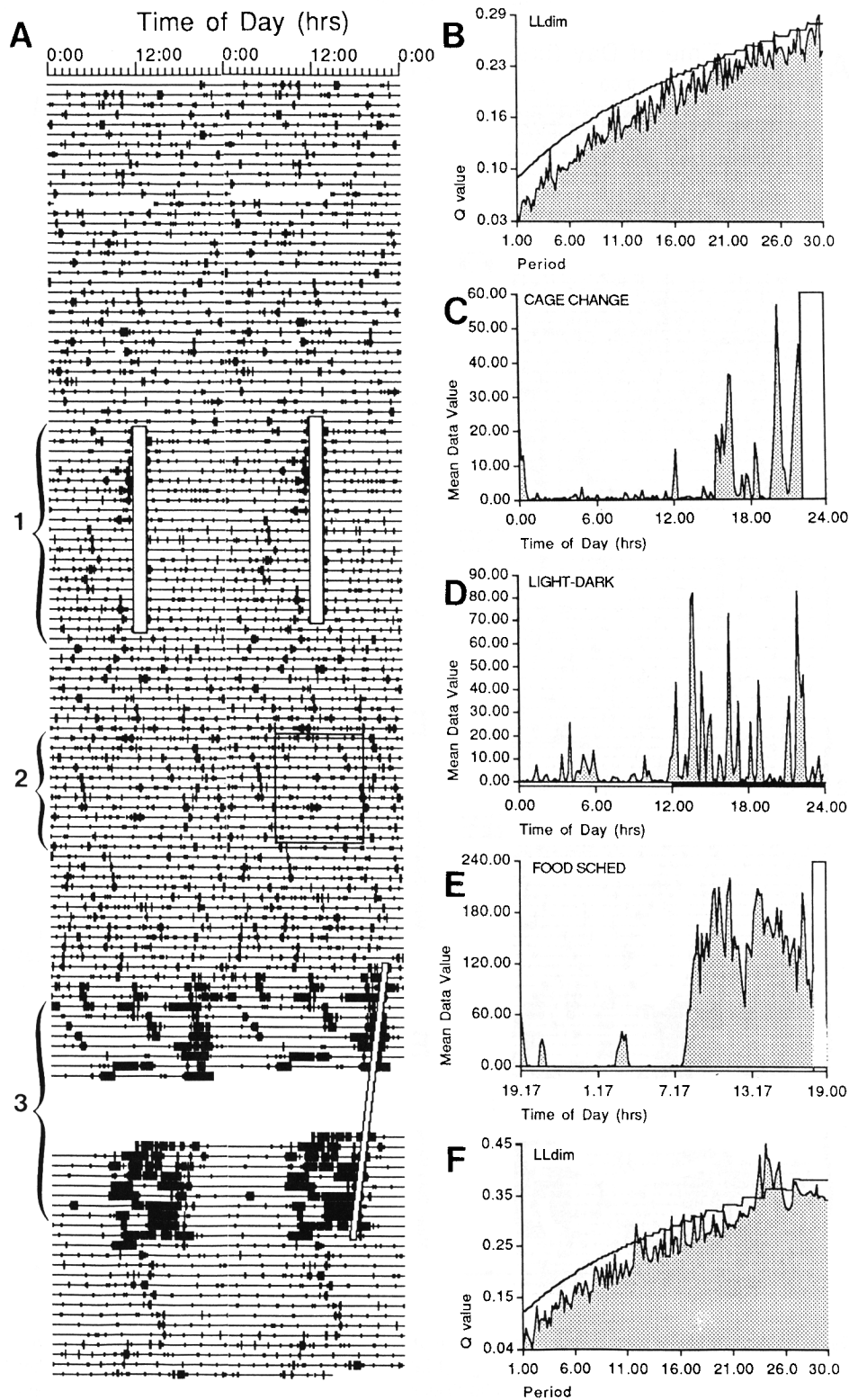


Figure 4. Activity charts for a hamster with a very large, complete suprachiasmatic nuclei ablation. (A: Double-plotted actogram recorded in dim light [LLdim], during a daily cage change schedule [Bracket 1], a 12:12-hr light-dark cycle [Bracket 2; solid lines block out the lights-on period], and a daily food restriction schedule [Bracket 3]. B: Periodogram analysis of the last 20 days of constant LLdim before cage change schedule; the stepping line represents the 95% confidence interval [*F* distribution]. C: Average waveform of the last 20 days of cage change schedule; the 2-hr cage change period is indicated by the open vertical bar. D: Average waveform of 11 days of the 12:12-hr light-dark cycle; lights-off period is indicated by the filled horizontal bar. E: Educated waveform [plotted at 23.83 hr] of the last 10 days of restricted feeding and drinking; the first 30 min of food availability is indicated by the open vertical bar. F: Periodogram analysis of the first 12 days of ad-lib food and water after the restriction schedule.)

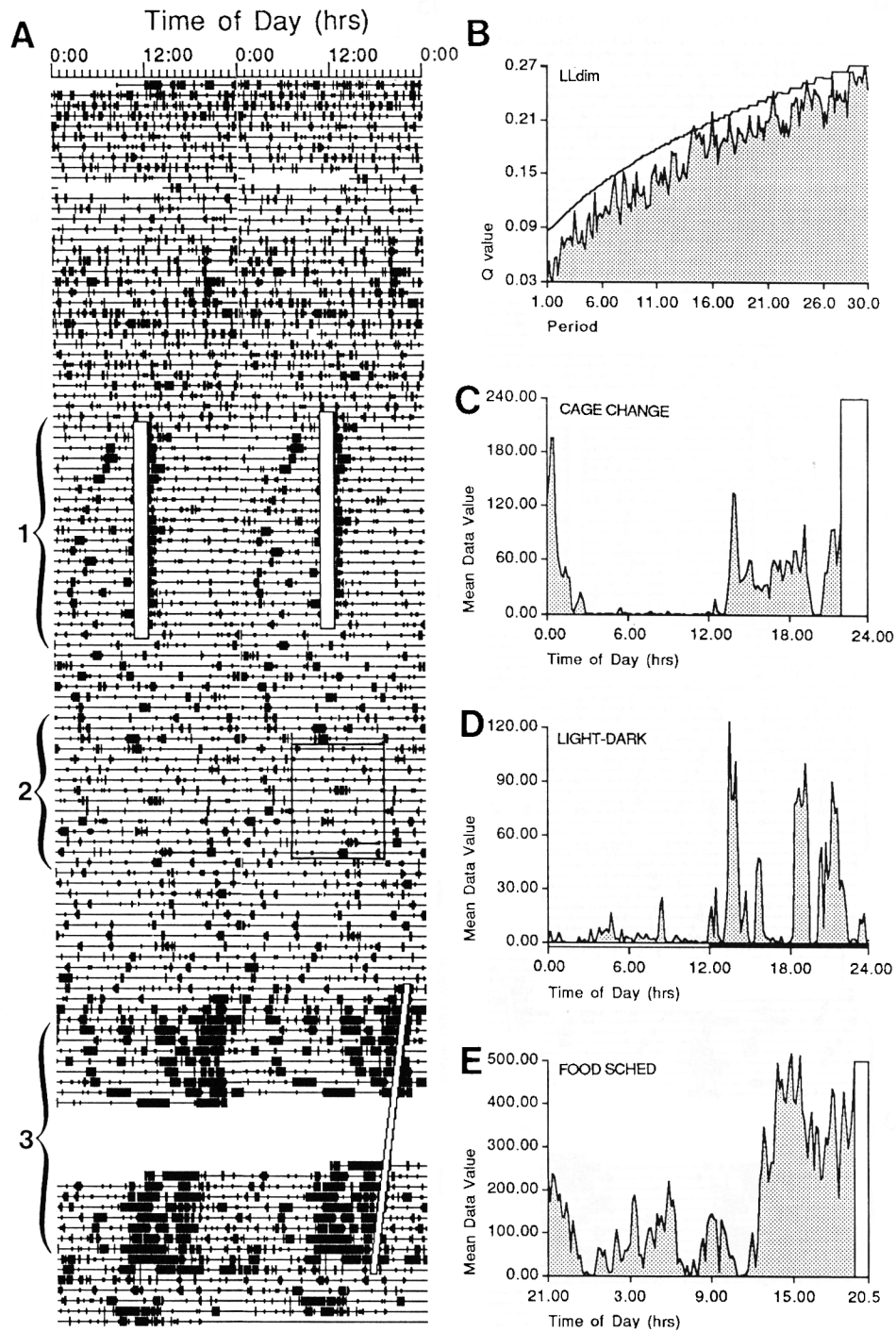


Figure 5. Activity charts for a hamster with a moderate-size, complete suprachiasmatic nuclei ablation. (A: Double-plotted actogram recorded in dim light [LLdim], during a daily cage change schedule [Bracket 1], a 12:12-hr light-dark cycle [Bracket 2; solid lines block out the lights-on period], and a daily food restriction schedule [Bracket 3]. B: Periodogram analysis of the last 20 days of constant LLdim before cage change schedule; the stepping line represents the 95% confidence interval [F distribution]. C: Average waveform of the last 20 days of cage change schedule; the 2-hr cage change period is indicated by the open vertical bar. D: Average waveform of 11 days of the 12:12-hr light-dark cycle; lights-off period is indicated by the filled horizontal bar. E: Educated waveform [plotted at 23.83 hr] of the last 10 days of restricted feeding and drinking; the first 30 min of food availability is indicated by the open vertical bar. F: Periodogram analysis of the first 12 days of ad-lib food and water after the restriction schedule.)

wheel running occurred at the beginning of the daily active phase, with a tendency in some cases (e.g., Figures 4E and 5E) for activity to decline in the hours immediately before feeding. When free feeding was reinstated, 3 of 6 animals (data for 2 hamsters were lost due to equipment failure) showed a free-running circadian activity component that was detectable both by eye and periodogram. This rhythm was far less robust than that observed during the feeding schedule.

Discussion

This study was designed to determine whether the SCN are necessary for nonphotic entrainment of circadian activity rhythms in the hamster. The results indicate that they are not; some hamsters that sustained unambiguous, complete SCN ablation displayed wheel-running rhythms that were synchronized to cage change and feeding schedules and that exhibited properties strongly suggestive of entrainment. The relations between these nonphotically entrained rhythms, the activity patterns observed in LLdim and LD 12:12, and lesion size will be elaborated.

Circadian Function After SCN Ablation: Constant Conditions

Complete SCN ablation invariably disrupts and can eliminate circadian organization of wheel running in hamsters but does not preclude the expression of rhythmic components in the circadian range. In the present study, 1 SCN-ablated animal displayed a 23.5-hr rhythm in LLdim before exposure to photic and nonphotic zeitgebers. Two other animals displayed multiple, partially independent rhythm components in the circadian range. Similar observations have been made previously and have been interpreted within a multioscillator framework (Rusak, 1977). Within this framework, the SCN are viewed as a compound, master pacemaker that couples (Rusak, 1977) or gates (Davis & Menaker, 1980) a population of oscillators located elsewhere. These oscillators can generate a coherent circadian rhythm of activity when exposed to a strong external synchronizing force (e.g., the LD cycle) but lack sufficient mutual coupling to sustain a stable, unimodal circadian population rhythm in constant conditions, which results in the appearance of multiple, partially independent rhythm components (e.g., animals Sx6 and Sx13).

Previous studies have reported a relation between SCN lesion size, particularly in the anterior SCN area, and the degree of circadian disruption (Davis & Gorski, 1984; Rusak, 1977). This suggests that the proposed non-SCN oscillators may be in the vicinity of the SCN and could perhaps be displaced SCN neurons that are spared by lesions intentionally confined to the Nissl stain-defined SCN. D. Edgar (personal communication, April 1, 1987) has noted that in squirrel monkey (*Saimiri sciureus*) brains "displaced" SCN neurons can sometimes be found embedded in the optic chiasm and that a few such cells may be sufficient to mediate circadian function in ablated animals. Other reports have also suggested that residual circadian function or recovery of function in ablated hamsters is associated with sparing of apparently just a few

histochemically defined SCN neurons (e.g., Harrington & Rahmani, 1990). It is thus conceivable that reports of circadian activity components in ablated animals reflect incomplete SCN ablation and inadequate histological evaluation; as Rusak (1989) has noted, it is not yet known which population of cells within the SCN (if not all such cells) are critical for rhythm generation; thus, it is unclear whether an SCN lesion is best evaluated by nissl stains or some more specific histochemical marker.

The present study took a blunt approach to this problem. A first group of hamsters was prepared with standard, well-localized SCN lesions, whereas a second group was subjected to much larger lesions, in some cases two to three times greater in volume. The results were in part counter to expectations. Most animals in both groups exhibited virtually random activity patterns with little or no evidence for residual circadian function. The 1 animal that exhibited a significant, short- τ , free-running rhythm did have a small, precisely localized SCN lesion that could have spared some SCN cells at anterior levels. However, the only other animals that showed obvious rhythmic components sustained very large lesions; in fact, the lesion in 1 of these 2 hamsters was the largest produced, spanned about 2.0 mm rostrocaudally and 1.5 mm dorsoventrally, and destroyed much of the MPOA, Pe, and PVN and some of the AH and Arc. One can only surmise from this that if circadian oscillators exist outside of Nissl-defined SCN, they are not confined to its immediate vicinity but may be distributed 0.5 mm or more away and are thus more likely to represent a unique cell population rather than residual, displaced SCN pacemaker cells.

Entrainment to Daily Cage Changes

In a previous study, wheel-running activity of 1 hamster with a complete SCN ablation exhibited synchrony to a daily 30-min opportunity to forage for seeds in an open field, which suggests that a nonphotically entrainable circadian mechanism may exist outside the SCN (Rusak et al., 1989). The present study confirms and extends this finding. Several hamsters that sustained complete SCN ablation showed a very similar pattern of synchrony to a daily activity schedule, in this case involving cage changes. As in the previous study (Rusak et al., 1989), spontaneous wheel-running preceded induced activity sessions, and in several cases, the rhythm disappeared rapidly at termination of the schedule. This pattern suggests an hourglass type mechanism that could work in the following way: The animal is quiescent for some hours after the daily induced activity period, perhaps eating and grooming intermittently. This may be viewed as a reasonable "homeostatic" adjustment to the vigorous activity (up to 3,000 wheel revolutions per hour) induced by cage changing. Normal levels of wheel running are then gradually reinstated and produce an apparent diurnal rhythm synchronized to the cage change schedule. At termination of the schedule, the pattern disappears abruptly because the rhythm is dependent on a sequence of behaviors and is not produced by a self-sustaining oscillator.

This explanation may fit some animals but is less convincing for others. In 6 hamsters with complete SCN ablation, one or

two discrete rhythmic components emerged from an arrhythmic baseline and synchronized to the activity schedule with a positive (anticipatory) phase in relation to the time of cage changing. When the schedule was terminated, these components persisted in some animals, usually with a short τ for at least a few cycles and in some cases for up to 10 days before destabilizing. These cases are more reasonably interpreted as reflecting entrainment of one or more self-sustaining oscillators. Rapid destabilization of the rhythms in constant conditions may reflect weak mutual coupling among these oscillators or rapid amplitude damping of individual oscillators.

An exact measure of lesion volume is difficult in animals with large ablations because surrounding areas may deform and "collapse" into the central zone of destruction. Nonetheless, a relation between lesion size and synchrony to the cage change schedule seemed apparent. The 4 hamsters that showed the most obvious persistence of rhythmic components had smaller lesions that were confined to the SCN and largely spared non-SCN tissue. In contrast, the 4 animals that showed no synchrony to the cage change schedule all had much larger lesions. Hamsters with synchronized patterns that fragmented rapidly at termination of the schedule also had moderate-to-large lesions. Unfortunately, no specific anatomical correlate of disruption could be identified. The larger lesions destroyed more tissue rostral, caudal, and dorsal to the SCN, but no nucleus was uniquely damaged in animals that showed little or no synchrony. The data appear to suggest that at least some nonphotically entrainable oscillators, or input-output pathways that are necessary for nonphotic entrainment, exist around the SCN area and that bigger lesions result in greater depletion of oscillators or pathways, which makes synchrony less likely and a coherent ensemble rhythm more difficult to sustain in the absence of an external synchronizing force.

The strong anticipatory phase of entrainment to the cage change schedule observed in the complete SCN-ablated hamsters differs markedly from the phase of entrainment noted in partial SCN-ablated hamsters observed in this study and in intact hamsters previously studied (Mrosovsky, 1988; Reeb & Mrosovsky, 1989). These latter animals typically entrained with the active phase of the daily rhythm beginning about 0.5–1.5 hr after the onset of the daily cage change (as determined on the day after the termination of such schedules). The reason for such a difference between intact and SCN-ablated animals is a matter of speculation. Within a multioscillator theoretical framework, system τ may depend on the total number of system oscillators or their phase relations because partial SCN ablations (which presumably deplete the total population) usually result in a shorter τ (cf Davis & Gorski, 1984). Shorter τ should in turn result in a more positive phase of entrainment (Pittendrigh & Daan, 1976). The complete SCN ablations produced in this study may have left only a small population of non-SCN oscillators, with a resulting short system τ and thus a positive phase of entrainment. Because of their general inability to sustain a free-running rhythm, it was not possible to evaluate τ in most of these animals. However, the few animals that did show a free-running rhythm after the cage change procedure did in fact have a short τ atypical of intact hamsters.

Synchrony to LD Cycle: Masking Versus Entrainment Interpretations

LD cycles have previously been shown to entrain residual circadian activity rhythms in SCN-ablated hamsters (Rusak, 1977). Although these entrained rhythms appeared abnormal, unstable, or both, a masking interpretation (direct suppression of activity by light and promotion of activity by dark, with no intervening entrained oscillator) could be discounted for some animals because transitions between rest-activity phases of their daily rhythms anticipated LD transitions, and the rhythms reset gradually rather than immediately after an LD shift. In the present study, 12 hamsters from Group 2 were exposed to LD 12:12 for up to 11 days, but none showed evidence of entrainment. In 2 animals, activity was distributed equally in the light and dark. Ten animals showed moderate-to-strong synchrony to LD, with reduced or no activity in the light and normal levels in the dark. However, this synchrony clearly reflects masking and not entrainment because transitions between rest-activity phases did not anticipate LD transitions and because the 24-hr rhythms disappeared immediately after resumption of LLDim. Whether photic entrainment would have emerged with continued exposure to LD seems unlikely, in view of the rapidity of photic effects observed in previous SCN ablation studies.

Failure to observe photic entrainment in this study (in contrast to other studies) probably reflects the extensive (albeit subtotal) damage to underlying optic chiasm observed in most animals and thus a more complete destruction of the retinohypothalamic tract (RHT) photic afferents that normally innervate hypothalamic oscillators within and, perhaps, outside the SCN. The data are consistent with the results of other studies that suggest a necessary role for the RHT in photic entrainment (e.g., Johnson et al., 1988). Neural pathways necessary for photic masking remain unknown. Clearly the SCN-RHT complex is not required. Preliminary observations indicate that the same is true in rats (Eastman, Mistlberger, & Rechtschaffen, 1983). The 2 animals in this study that did not exhibit masking sustained the greatest damage to the optic chiasm. These lesions may have effectively reduced total retinal afferents projecting past the optic chiasm such that the light intensities used were inadequate to affect behavior.

It is interesting that the nonphotically entrainable oscillators that are proposed to mediate synchrony to the cage change schedule observed in some animals were not entrained by the rest-activity cycle created by photic masking. This is perhaps not surprising, given the low levels of activity expressed even in the dark period of the LD cycle. Entrainment to activity schedules is thought to require fairly intense activity, although a formal dose-response curve is not yet available.

Entrainment to Restricted Food and Water Availability

The most dramatic effect observed in this study was the uniform response of complete SCN-ablated hamsters to the food and water restriction schedules. All 8 animals tested displayed stable, high amplitude wheel-running rhythms with a positive (anticipatory) phase of entrainment to feeding time. This result is consistent with a preliminary report that SCN-

ablated hamsters anticipate a daily palatable meal (Abe & Rusak, 1990). The robust anticipatory rhythms observed here are best interpreted as reflecting entrainment of self-sustaining oscillators because 3 of 6 animals showed evidence of a persisting circadian rhythm that free-ran from the apparent phase of entrainment when the feeding schedule was terminated. In addition, the waveform of the entrained rhythm does not fit the most likely prediction of an hourglass interpretation, which would be that wheel running, which is stimulated by food deprivation, would be maximal just before mealtime, when food deprivation is greatest. Instead, peak wheel-running rates were consistently observed at the beginning of the active phase of the rhythm, often 6–10 hr before mealtime.

Food restriction would superficially appear to be a stronger zeitgeber for nonphotically entrainable oscillators than is activity induced by cage changes. This may be more apparent than real. Food deprivation is well known to promote activity and may combine with nonphotically entrainable oscillators to enhance the expression of the active phase of the nonphotically entrained rhythm. Food deprivation might similarly enhance the expression of rhythms entrained to cage change schedules.

The adaptive significance of entrainment to feeding time in hamsters is at present unclear. Abe and Ebihara (1990) have observed such entrainment in intact animals, thus it is not a peculiarity of an experimentally reduced circadian system. Feeding time is a potent zeitgeber for circadian rhythms in other rodents, particularly the rat, and the mediating oscillators are also located at least in part outside of the SCN and nearby hypothalamic areas, although where is unknown (Mistlberger, 1990; Mistlberger & Rusak, 1988). For an opportunistic, omnivorous feeder such as the rat, it seems likely that a food entrainable pacemaker would afford a survival advantage in coordinating foraging behavior with external periodicities of food availability. The hamster, however, is characteristically a prodigious food hoarder; it forages nocturnally but feeds throughout the day from hoarded stocks that may exceed 2 weeks in supply. Moreover, hamsters do not exhibit hyperphagia after food deprivation and thus survive very poorly under limited duration (as opposed to limited amount) schedules. Even a 12-hr food access schedule is quickly fatal (Silverman & Zucker, 1976). It would thus seem unlikely that the hamster would routinely (if ever) use a food-entrainable pacemaker to guide foraging because it may never experience 24-hr periodicities of food intake. The food-entrainable component of rodent circadian systems may perhaps be viewed as vestigial in species such as the hamster that rely on food hoarding as opposed to food binging to maintain an adequate short-term energy store.

Alternatively, food-entrainable oscillators in the hamster may represent a general mechanism for entrainment or rapid phase adjustments to any significant nonphotic stimuli, such as preferred foods, predators, or mates (social cues). In hamsters, these oscillators may thus have a significance beyond the generation of rhythms in response to the rather artificial restricted feeding schedule used in this study. Whether feeding, activity, and potential social zeitgebers entrain the same oscillators is presently unknown, although this would clearly be the most economical solution to the general problem of nonphotic entrainment. It has been argued previously that

other rodents may differ in this respect; rats, for example, show a very weak entrainment to daily activity schedules that is probably not mediated via food-entrainable oscillators (Mistlberger, 1991).

The major conclusions of this study can be summarized as follows. The SCN contains a circadian pacemaker necessary for normal expression of circadian rhythms in constant conditions. Complete destruction of the SCN–RHT complex eliminates photic entrainment but not photic masking and some other circadian functions; ablated hamsters may exhibit unstable rhythm components in LLdim and can be entrained by nonphotic stimuli such as daily cage changes or restricted feeding schedules. In view of the large size of the lesions, at least some oscillators mediating these rhythms must be located at a distance from the SCN and are likely to represent a distinct cell population. However, persistence of nonphotically entrainable rhythms was weaker or absent in hamsters with larger lesions, which suggests that some nonphotically entrainable oscillators reside in the hypothalamus close to the SCN and perhaps also within the SCN. The specific cell populations that generate these rhythms, and the pathways by which they are entrained, remain to be identified.

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Zahn-Waxler Appointed New Editor, 1993-1998

The Publications and Communications Board of the American Psychological Association announces the appointment of Carolyn Zahn-Waxler as editor of *Developmental Psychology*. Zahn-Waxler is associated with the National Institute of Mental Health. As of January 1, 1992, manuscripts should be directed to

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Manuscript submission patterns make the precise date of completion of the 1992 volume uncertain. The current editor will receive and consider manuscripts through December 1991. Should the 1992 volume be completed before that date, manuscripts will be redirected to the incoming editor for consideration in the 1993 volume.