Circadian Entrainment to Temperature, But Not Light, in the Isolated Suprachiasmatic Nucleus

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INTRODUCTION

Daily changes in behavior and physiology are often endogenously driven by circadian pacemakers. To be useful to the organism, these pacemakers synchronize (entrain) to environmental cycles and accurately signal time-of-day from cycle-to-cycle over the physiological range of temperatures. Most organisms depend on fluctuations in environmental lighting to entrain to local time. In mammals, photic information from the eyes entrains the suprachiasmatic nuclei (SCN), the master circadian pacemaker in the brain (Klein et al. 1991; Ralph et al. 1990). Many organisms are also entrained by temperature cycles which generally fail to or weakly affect mammalian circadian rhythms (reviewed in Rensing and Ruoff 2002). The SCN may also entrain directly to light. In nonmammalian vertebrates, circadian photoreceptors have been localized to a variety of neural and nonneural tissues (Foster et al. 1994; Whitmore et al. 2000). In mammals, at least two gene products highly expressed in the retina and SCN have been implicated in photoreception (Miyamoto and Sancar 1998; Van Gelder et al. 2003). Finally, a report that pupillary illumination shifts human circadian rhythms (Campbell and Murphy 1998) has prompted a reexamination of the potential for extraocular photoreception in mammals (Wright and Czeisler 2002; Yamazaki et al. 1999). These observations led us to ask whether the isolated SCN can entrain to temperature or light cycles. Perhaps the least understood characteristic of all circadian pacemakers is their ability to keep accurate time over a range of temperatures. Temperature has little effect on the period of circadian rhythms in poikilotherms (Kondo et al. 1993; Menaker and Wisner 1983; Pittendrigh 1981; Underwood 1985) and heterotherms (Grahn et al. 1994; Menaker 1959). Temperature compensation of circadian period in homeotherms has been demonstrated in vitro for the avian pineal (Barrett and Takahashi 1995; Zatz et al. 1994), the mammalian retina (Tosini and Menaker 1998), and the rat SCN (Ruby et al. 1999). It is unclear whether the mechanism is cell autonomous or depends on cell-cell interactions.

METHODS

Animals

We assessed the effects of light or temperature cycles on intrinsic rhythmicity in the SCN. Tissue for the entrainment experiments was harvested from transgenic rats (Japanese Wistar) expressing 6.7 kb of the mouse Per1 promoter driving the firefly luciferase gene. Details on the generation of the Per1-luc rats have been previously published.
(Yamazaki et al. 2000). Tissue for the temperature compensation experiments was harvested from mice (C57BL/6J × BALB/cJ intercross background). Animals were maintained at Washington University under a 12:12 h light:dark schedule. All procedures were approved by the University’s Animal Care and Use Committee and conformed to National Institutes of Health guidelines.

**Multielectrode recording**

Dispersed cell cultures were prepared from neonatal mice (0- to 5-days-old). Pups were decapitated without anesthesia. Coronal sections of the brain, 300-μm-thick, were made using a vibroslicer. Cylindrical punches (410-μm-diameter) of unilateral SCN from five to eight pups were pooled, dispersed, and grown according to published methods (Herzog et al. 1998). Viable cells were plated at approximately 2,000 cells/mm² onto a multimicroelectrode plate (Dr. Guenter Gross, Univ. North Texas, Denton, TX) and incubated at 37°C in 5% CO₂, 95% air for 2–3 wk. Culture medium (pH 7.2) consisted of DMEM (Invitrogen No. 31053-028, Invitrogen, Carlsbad, CA) supplemented with B27 (Invitrogen No. 17504-010), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen, No. 15140-122), and 10% neonatal calf serum (Invitrogen No. 16010-159). After about 2–3 wk in vitro, cultures were transferred to a recording chamber with 1 ml of recording medium. Recording medium was identical to culture medium except that it was supplemented with 10 mM HEPES and NaHCO₃ levels were reduced from 1.2 to 0.3 g/l. Half of the medium was exchanged about every 2.5 days. We simultaneously recorded extracellular voltage signals from two cultures for up to 10 days, and averaged the signals. The data were filtered at 300 kHz, and the frequency of spontaneous activity was set to 3 kHz minimum sample rate. We simultaneously recorded extracellular voltage signals from two cultures for 2–5 days. Signals from up to eight electrodes were simultaneously amplified with an overall gain of 10,000–20,000 and band-pass filtered at 300–3,000 Hz (Cyberamp 380 and 401, Axon Instruments, Union City, CA). The signals were displayed on oscilloscopes and digitized (12-bit resolution, 15-kHz minimum sample rate) on an IBM 586-type personal computer using custom software. Every 10 min, the number of spikes with identical amplitude and duration was totaled and stored. Using off-line analysis methods similar to published methods (Meister et al. 1994), we assigned action potentials of similar amplitude and duration to individual cells and counted impulse frequency. The shape of the impulse and presence of a clear refractory period following the impulse were used as criteria for single-unit activity.

Two separate systems were used to control the culture temperature. In both systems, the culture lid was heated by a constant current flowing through a power resistor. This prevented condensation on the lid. Cultures were also heated from below either by a pair of power resistors heated by a temperature controller (model TC202A, Harvard Apparatus, Holliston, MA) or by water circulating through the recording stage (model E200, Lauda, Westbury, CT). Temperature inside the recording chamber was recorded with a thermocouple (PT-6 with Thermes-16, Physitemp Instruments, Clifton, NJ). All temperature sensors were calibrated against a NIST-calibrated thermometer (Fisher Scientific No. 15043A). Long-term recordings showed that the temperature of the culture medium did not fluctuate with time of day and ranged by <0.25°C from the set point.

**Real-time Per1-luc measurement**

Explant cultures from neonatal (1–8 days after birth) and juvenile rats (19–39 days old) were made from Per1-luc rats approximately 1 h before dusk. Juvenile rats were anesthetized with CO₂ and decapitated and their brains were rapidly removed. Pups <8 days of age were rapidly decapitated without anesthesia. Coronal sections of the brain, 400-μm-thick, were made with a vibratome. Bilateral or unilateral SCN explants were placed on Millicell membranes (PICM0RG50, Fisher Scientific) with 1 ml of recording medium supplemented with 0.1 mM of beetle luciferin (No. E1601, Promega, Madison, WI). These cultures were sealed in a 35-mm petri dish with a coverslip and vacuum grease. Bioluminescence was recorded within 2 h after surgery at 1-min intervals with a photomultiplier tube (HCl135–11M0D, Hamamatsu) as previously described (Geusz et al. 1997).

Per1-luc cultures were maintained and recorded in large, air-jacketed incubators (6.67 cubic ft, model 1535, WVR, West Chester, PA). Temperature ranged by <0.3°C from the set point without circadian variation. Temperature cycles were imposed by changing the incubator set point. Culture temperature changed at a rate of 2.5°C per hour so that a 3°C shift was complete within 1.2 h and a 1°C shift was complete within 0.4 h.

 Cultures exposed to light cycles were maintained in black boxes with black felt lids. Exposing the culture to light by removing the felt from a box had no effect on the temperature inside the box (data not shown). A single, 8 W fluorescent bulb (s928, Sylvania) covered with a diffuser produced 2.6–6.1 × 10¹⁵ photons cm⁻² s⁻¹ (approximately 150 lx) at the level of the cultures and was attenuated 1,550-fold by the black felt. The majority of emitted light (88%) was between 500 and 700 nm with peaks typical of a mercury lamp at 547 and 578 nm. Approximately 7% of the total emission was between 300–450 nm, with smaller peaks at 404 and 436 nm. Light intensity was calibrated using a radiometer (Li185b, LI-COR, Lincoln, NE) and sensor (Li190SB Quantum). The 6-h advance in the light schedule was accomplished by advancing the time of lights-on, leading to one short, 6-h night. The 6-h delay was accomplished by delaying the time of lights-off, resulting in one long, 18-h day.

**Data analysis**

The phase of the bioluminescence rhythms was determined as previously reported (Abe et al. 2002). Briefly, a 24-h running average was subtracted from the raw data to reduce changes in the baseline that occur over subsequent days. Figure 1A shows representative raw data and Figs. 2A, 3A, and 5, A and B, show representative detrended data. Then a 3- and a 24-h running average were calculated from the detrended data set. The phase of peak bioluminescence between the crossings of these two smoothed lines was used as the phase marker for each cycle of the recorded rhythms. All times were reported in “Zeitgeber Time” (ZT) relative to animal’s prior light cycle such that ZT0 was the time of light onset in the animal colony (7 AM) and ZT12 was the time of light offset (7 PM).

 Cultures were defined as “entrained” when the phase of their first peak following an entraining cycle was more than one SD unit from the control condition, within one SD unit of the other cultures within the treatment, and the phase of subsequent cycles showed a stable free-running period for at least three more days. Period of Per1-luc cultures was determined as the average time between bioluminescence peaks over at least 4 days. Phase and period differences were analyzed by two-tailed Student’s t-test using Origin6.1 (OriginLab, Northampton, MA). Free-running periods of firable rate records from individual cells were quantitatively assessed from at least 3 days of data by a fast Fourier transform nonlinear least-squares method (FFT-NLLS; Herzog et al. 1997). On completion, FFT-NLLS provided period estimates with 95% confidence limits. The temperature coefficient was calculated by using the equation $Q_{10} = \frac{q_{t+1}}{q_t}^{10(T_{t+1} - T_t)}$, where $q$ is the cell’s period and $T$ is temperature.

**RESULTS**

**Temperature cycles entrain SCN rhythmicity in vitro**

To address whether physiological fluctuations in temperature can alter SCN pacemaking, we exposed rat SCN explants to eight 1.5°C cycles roughly in anti-phase to the normal rhythm in brain temperature (cf. Gao et al. 1995). SCN cultures...
synchronized their rhythmicity to these cycles (Fig. 1A). Over the first 3–5 days, the rhythm in Per1-luc expression gradually advanced until the peak occurred approximately 0.5 h after the cool-to-warm transition (Fig. 1B). This phase relationship was stable and seen in all cultures (n = 6 of 6 entrained). Compared with Per1-luc rhythmicity in the SCN in vivo (Yamaguchi et al. 2001) and on the first day in vitro (Abe et al. 2002; Stokkan et al. 2001; Yamazaki et al. 2000), the temperature cycle advanced these cultures by 9.5–11 h.

When the temperature was then held at 36.8°C for an additional 5 days, rhythms free-ran from the entrained phase with an average period of 24.8 ± 0.4 h. Control cultures maintained at 36.8°C for 10 days showed no signs of entrainment and had a period of 25.2 ± 0.2 h (mean ± SE). The phase difference between the control and treated cultures was significant after the first temperature cycle (t = 5.8, P < 0.000001). These data indicate that the SCN can entrain to physiological temperature oscillations.

This synchronization was independent of age. Cultures from 1- and 19-day-old (P1 and P19) rats showed similar phase relationships to the temperature cycle (peaking at ZT 0.7 ± 0.3 and 0.3 ± 0.5, respectively, n = 3 in each condition; t = −0.67, P = 0.54). The initial peak, prior to temperature cycling, of the P19 SCN was at ZT 10.0 ± 0.2, as has been found in rats aged 30–40 days (Abe et al. 2002). The free-running periods of the neonates and juveniles were similar (24.7 ± 0.9 and 25.3 ± 0.2 h, respectively). Thus entrainability to temperature develops early in the ontogeny of the SCN.

Left and right SCN similarly entrained. No differences were found in their initial phases prior to temperature cycles (ZT 10.0 ± 0.4 for left and 10.2 ± 0.5 for right, t = 0.24, P = 0.81, n = 12 left and 13 right SCN) or their phases after temperature entrainment (ZT 0.7 ± 0.3 for 3 left SCN, 0.3 ± 0.5 for 3 right SCN, t = −0.55, P = 0.61).

Amplitude of the temperature cycle affects the phase of entrainment

The phase angle of entrainment, measured as the delay between the peak of the biological rhythm and the peak of the entraining rhythm, is generally a function of the strength of the environmental timing cue. Previous work showed that 3°C pulses shift the firing rate rhythm in the SCN by ≈3 h (Ruby et al. 1999). To test whether this stimulus could entrain the SCN to a different phase compared with a 1.5°C cycle, we exposed unilateral SCN to three 12:12 h cycles of 34:37°C. Daily temperature rose approximately 3 h earlier than the brain’s natural oscillation. Rhythms in the treated SCN peaked
approximately 0.5 h before the cool-to-warm transition and were approximately two to four times the amplitude of untreated cultures (Fig. 2A). Relative to control cultures, treated SCN were advanced by an average of 1.3 h during the temperature cycles and 3.4 h on the first cycle of the free-run (n = 3 of 10 entrained vs. 8 controls). The free-running rhythms peaked at a phase approximately 1–2 h earlier than they had during the temperature cycles. This “masking” effect on our ability to assess the true phase of the intrinsic oscillator in temperature cycles was similar in 1.5 and 3°C cycles (see also Fig. 1B, where the initial peak in the free-run was advanced relative to peak phase in the prior temperature cycle). Those cultures that failed to entrain all showed “transient entrainment,” where their first peak in constant conditions appeared as if entrained, but subsequent cycles jumped back to the phase of untreated controls. Importantly, relative to the time of daily warming, entrained cultures in the 3°C cycle peaked approximately 2 h prior to cultures in 1.5°C cycles (compare Fig. 1B and 2B). Thus the phase angle of entrainment of SCN rhythmicity depended on the amplitude of the temperature cycle.

To test whether a 1°C cycle is also sufficient to entrain, cultures were exposed to 2.5 cycles of 12:12 h 35.7:36.7°C cycle. Left and right SCN were dissected and randomly assigned to two groups. After 2 days at constant temperature, explants experienced a daily temperature increase at ZT 9 (n = 8) or 15 (n = 9). During the temperature cycles, the rhythms moved their daily maximum toward the transition from cool-to-warm (Fig. 3). However, despite experiencing schedules that were 6 h apart, cultures failed to significantly shift relative to each other (n = 0 of 17 entrained). This again suggests that temperature has acute effects on Per1-luc expression that can mask the true state of the underlying oscillator and that synchronization to a 3-h advance or delay requires temperature fluctuations larger than 1°C or more than 2.5 entraining cycles.

**Circadian periodicity is temperature compensated in individual SCN neurons**

Previous work showed that the timing of daily peaks in SCN firing rate changes little over a range of physiological temperatures (Ruby et al. 1999). Because the mechanisms underlying this temperature compensation of period are unknown, we sought to determine whether this property, ubiquitous among circadian pacemakers, is intrinsic to individual cells. Because mice can exhibit large changes in body temperature during torpor, we chose to record from mouse SCN neurons from 24 to 37°C. SCN neurons plated at low density on multielectrode arrays expressed circadian rhythms in firing rate over a range of 13°C (Fig. 4A). As previously reported, we found neurons in the same culture with different circadian periods, indicating they were independently oscillating (Herzog et al. 1998; Honma et al. 1998; Liu et al. 1997a; Welsh et al. 1995). Firing patterns were recorded from 9 neurons at multiple temperatures in five cultures. The circadian period of most neurons increased slightly with warming (n = 8 of 9 neurons; Fig. 4B). Individual cells had $Q_{10}$ values of 0.94 ± 0.04 (mean ± SE, range from 0.68 to 1.07). Including neurons for which we were able to estimate the period at only one temperature (n = 60 cells in 15 cultures), the average periods during 24, 30, 33, and 37°C were 23.6 ± 0.5, 22.1 ± 0.7, 23.8 ± 0.6, 23.6 ± 0.4 h, respectively (Fig. 4C). The $Q_{10}$ of the ensemble data were 0.99 from 24 to 37°C, indicating that circadian period of single neurons is temperature compensated.

The percentage of rhythmic neurons decreased with temperature (Table 1). Only 19% of the recorded cells expressed circadian rhythms in firing rate at 24°C, in contrast to 50% at 30°C, 28% at 33°C, and 68% at 37°C. Indeed, 10 of the 21 cells recorded at 24°C were arrhythmic at 24°C, but rhythmic at a warmer temperature (Fig. 4B). Although all cells had a daily decline in firing to near silence, their peak discharge rates were heterogeneous even at a given temperature (Fig. 4A) so that there was no significant effect of temperature on maximum ($F = 1.36, P = 0.27$) or median firing rates ($F = 0.44, P = 0.72$; Table 1). These results suggest that although the pacemaker can keep accurate time over the range of physiological temperatures, its ability to rhythmically regulate firing rate is compromised at lower temperatures.
Light cycles fail to directly entrain the cultured SCN

Previous reports suggested the possibility that the brain is directly light sensitive (Blackshaw and Snyder 1999; Marchant and Morin 2001; Miyamoto and Sancar 1998; Wade et al. 1988) and at least one report specifically implicated the SCN (Lisk and Kannwischer 1964). To test whether the SCN is intrinsically light sensitive, we exposed SCN cultures to a 6 h advanced or 6 h delayed light schedule. Left or right SCN experienced lights on at either ZT 6 or 18 for 5 days and were then maintained in constant darkness for an additional 5 days. We recorded bioluminescence from the cultures immediately following the fifth dusk. Figure 5 shows that light had no effect on the phase of rhythmicity, with SCN in the advanced and delayed treatments reaching their first peak in constant darkness at between 4.7 and 5.1 1 h after lights on in the animal colony (n/H11005 5 SCN in each condition; t/H11005/H11002 0.25, P/H11005 0.81).

No effect of light was noted in SCN from 2- or 31-day-old rats (n/H11005 4 and 6, respectively) in this experiment. Results from three further experiments on rats at ages 6 (n/H11005 5), 9 (n/H11005 5), and 339 days (n/H1105 6) were similar (data not shown). Thus the SCN failed to directly entrain to light cycles that would have entrained the animal.

DISCUSSION

Environmental temperature has been considered to be an effective timing cue in poikilotherms, but ineffective in mammals including rodents and primates (DeCoursey 1960; Sulz-...
man et al. 1977). Rhythmicity in the mammalian SCN was recently reported to be unaffected by changes in environmental temperature (Brown et al. 2002). However, we found that daily temperature fluctuations of as little as 1.5°C reliably entrain the isolated SCN. This suggests that, while physiological mechanisms buffer brain temperature against environmental cycles, the normal rhythms in brain temperature are sufficient to entrain the SCN in vivo.

The phase relationship between SCN and temperature rhythms is similar in vivo and in vitro. Rats show a sharp increase in brain temperature of 1–2°C approximately 1 h prior to the onset of locomotor activity (reviewed in Gao et al. 1995; Refinetti and Menaker 1992). In vivo, peak Per1-luc expression in the rat SCN occurs at approximately ZT 10, 1 h prior to the increase in brain temperature (Abe et al. 2002; Yamazaki et al. 2000). Per1 levels in mice are similarly regulated (Wilsbacher et al. 2002; Yamaguchi et al. 2001). When exposed to 1.5°C temperature cycles in vitro, Per1-luc in the SCN peaks approximately 1 h before the daily rise in temperature (Fig. 1B). This phase angle of entrainment is advanced in 3°C temperature cycles so that peak bioluminescence occurs about 3 h prior to the daily rise in temperature (Fig. 2B). It is reasonable to postulate, therefore, that daily cycles in brain temperature entrain the SCN, placing temperature on the output of and input to the SCN. Such a feedback loop could serve to increase amplitude or cycle-to-cycle stability and perhaps to communicate phase to peripheral oscillators. Indeed, daily changes in body temperature have recently been shown to alter the rhythmic expression of genes in the liver (Brown et al. 2002). Importantly, this same study found that environmental fluctuations from 24 to 37°C had no effect on the SCN, indicating that this deep brain structure is well buffered against ambient heating and cooling.

Although daily warming can shift the phase of the SCN, long-term recordings of firing rate revealed that the circadian period of individual SCN neurons is temperature compensated (Fig. 4). As found for circadian rhythms in a wide variety of organisms (Bunning 1973; Pittendrigh 1954; Ruby et al. 1999; Sweeney and Hastings 1960), the period changes by <10% with a 10°C change in temperature. In contrast, most biochemical reaction rates typically double or triple over a 10°C range. We found SCN neurons cultured at low density express different circadian periods from one another, as has been reported by others (Welsh et al. 1995). As temperatures increased, most neurons showed slightly longer periods, but continued to express periods distinct from others in the same culture. Taken together, these results indicate that each neuron is likely an independent, temperature-compensated circadian oscillator.

The mechanisms underlying temperature compensation are unknown. Temperature compensation is altered by mutations in genes that play a central role in rhythm generation in mammals (Tosini and Menaker 1998), fungi (Loros and Fieldman 1986), and flies (Konopka et al. 1994). It may be that temperature differentially regulates the abundance (or activity) of different “clock gene products,” having direct, compensatory effects on transcriptional activators and repressors. Alternatively, a mechanism outside the central core may act as a governor controlling pacemaker rate. In either scenario, a pair of reactions might speed up with increasing temperature, a reaction countering the effects of the other (Hall 1997). It will be important to follow up the observation that Per1-luc expression was higher at lower temperatures with experiments that directly address the effects of temperature on clock gene activity.

There are temperature limits to circadian rhythmicity. Daily hypothalamic temperature in rats typically ranges between approximately 36.8 and 38.6°C when ambient temperature is between 10 and 30°C (Abrams and Hammel 1963; Gao et al. 1995; Gordon 1990). The results here showed that, from 24 to 37°C, the period of the mouse SCN was intrinsically temperature compensated, but the percentage of rhythmic neurons decreased at temperatures below approximately 33°C. This is consistent with the observation that, from 37 to 25°C, the amplitude of ensemble firing in the rat SCN decreased by approximately 70% after 1 day and rhythmicity was lost after 3 days in vitro (Ruby and Heller 1996). Also, consistent with previous reports (Burgoon and Bouliant 1998), we found that SCN neurons either showed decreased or unchanged firing as they cooled. SCN neurons, however, are heterogeneous in their firing rates (e.g., peak firing rates in this study ranged from 0.1 to 12.1 Hz across cells at 37°C). This variability may explain why the tendency toward decreased firing at lower temperatures did not reach significance (Table 1).

The effects of temperature on SCN function are also species specific (cf. Ruby and Heller 1996). Brains in organisms such as mice, bats, and ground squirrels can drop below 25°C during bouts of torpor or hibernation and may still express circadian rhythms in body temperature (Hut et al. 2002; Ruby et al. 2002). Recently, Larkin et al. (2002) provided compelling evidence that the circadian regulation of brain temperature continues, albeit at much reduced amplitude, in the hibernating squirrel SCN at ambient temperatures as low as 9°C. These animals, however, lost their circadian rhythm in slow-wave sleep. The SCN of some hibernators may thus be adapted to regulate selected rhythms at lower temperatures. In general, reducing brain temperature below normal appears to suppress SCN regulation of rhythmic outputs such as firing rate, perhaps by reducing the amplitude of the core circadian oscillator.

In contrast to the direct effects of temperature on SCN timing, light cycles failed to entrain SCN cultures (Fig. 5). Rodents can entrain to 6-h shifts in their light schedule within 5 days (cf. Yamazaki et al. 2000) and to intensities above approximately 10^{11} photons-cm^{-2}s (Nelson and Takahashi 1991). In our hands, lights as bright as 6 × 10^{14} photons-cm^{-2}s cycling for 5 days had no effect on SCN rhythmicity. This is in agreement with several studies showing that the eyes are necessary for photic entrainment in mammals (cf. Yamazaki et al. 1999). Genes expressed in both the SCN and the retina, similar to Cryptochrome 1 and 2, may play the same role in the two tissues (e.g., rhythm generation), but are unlikely to be involved photoreception in the SCN.

Little is known about how temperature entrains the SCN. The phase response curve to 3°C temperature pulses by Ruby et al. (1999) predicted that the SCN can shift a maximum of 3 h per day. Consistent with this, we found that the SCN took 3 days to advance approximately 10 h in a 1.5°C temperature cycle (Fig. 1B). It is possible that temperature acts on a neurotransmitter system within the SCN to synchronize cells to a new schedule. Liu and Reppert (2000) showed that daily pulses of GABA will synchronize individual SCN neurons in vitro so that the GABA pulse falls during the cells’ early subjective night, producing a daily phase delay. Ruby et al.
(1999) found that heat pulses at this time also delay SCN rhythmicity. This agrees with our finding that the SCN entrained so that the daily rise in temperature occurred during the early subjective night (approximately 1–2 h after the peak of Per1::luc expression; Fig. 1B). However, the phase response curve to GABA included a large delay zone during the subjective day (Liu et al. 1999). Instead, temperature steps may act directly on intracellular events to shift individual cells. Temperature might, for example, modulate CLOCK-BMAL1 heterodimer formation through changes in cellular metabolism and redox state (Rutter et al. 2002). In the fungus, Neurospora crassa, warming potently resets levels of frequency (FRQ) protein which resets the clock (Liu et al. 1998). Temperature also regulates the threshold at which FRQ protein suppresses its own transcription and the abundance of two forms of FRQ, by favoring different initiation codons at different temperatures (Liu et al. 1997b). How temperature is transduced to the nucleus is largely unknown, but it is now clear that one or more intracellular mechanisms enable the circadian clock to keep accurate time over a range of temperatures and to synchronize to temperature cycles.

The role of temperature in circadian entrainment may be highly conserved across phyla and fundamental in mammals. Because light at night causes an increase in brain temperature (Song and Rusak 2000), the two stimuli may act synergistically to shift the clock. Temperature entrainability develops early within the SCN, even before rhythms in body temperature (Melanie et al. 1986). It is therefore conceivable that maternal presence, heavy exercise, fever, and treatments that alter brain temperature could perturb normal circadian timekeeping (Jilge et al. 2000; Luker et al. 2000; Satinoff 1972; Satinoff et al. 1991). A better understanding of how temperature resets the pacemaker will likely lead to a better understanding of the mechanisms that generate and synchronize circadian rhythmicity over the physiological range of temperatures (Krisnan et al. 2001; McWatters et al. 2000; Merrow et al. 1999).

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DISCLOSURES

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