Temperature Sensitivity of the Suprachiasmatic Nucleus of Ground Squirrels and Rats in vitro

Norman F. Ruby[1] and H. Craig Heller

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

Abstract Temperature compensation of circadian rhythms in neuronal firing rate was investigated in the suprachiasmatic nucleus (SCN) of ground squirrels and rats in vitro. A reduction in SCN temperature from 37 to 25°C reduced peak firing rates by > 70% in rats but only by ≈ 21% in squirrels; trough firing rates were marginally altered in both species. In the rat SCN at 25°C, the peak in neuronal activity decreased progressively on successive days and circadian rhythms no longer were present by Day 3. There was a 37% reduction in the number of single units detected and an increase in the temporal variability of peak firing rates among individual rat SCN neurons at low temperature. By contrast, single units were readily detected and circadian rhythms were robust in squirrels at 37 and 25°C; a Q_{10} of 0.927 was associated with a shortening of tau by 2 h and a 5-h phase change after only 48 h at low temperature. These results suggest that temperature can have a substantial impact on circadian organization in a mammalian pacemaker considered to be temperature compensated.

Key words circadian rhythm, temperature compensation, Q_{10}, brain slice, single unit, hibernation

INTRODUCTION

Accurate time measurement by biological clocks requires that daily, seasonal, and acute fluctuations in temperature not significantly influence circadian pacemaker period (Pittendrigh, 1974; Pittendrigh and Daan, 1976a). This supposition is supported by the numerous observations of temperature compensation of circadian pacemakers in several phylogenetically diverse organisms including several mammalian species (Berger et al., 1992; Kondo et al., 1993; Lindberg et al., 1971; Menaker and Wisner, 1983). Circadian pacemakers in these organisms are considered to be temperature compensated because the periods (i.e., tau) of their circadian rhythms (CRs) have Q_{10}s between 0.85 and 1.15. This is in marked contrast to the high temperature sensitivity of most biological processes that have Q_{10}s between 2 and 3. Temperature compensation does not mean, however, that temperature has no effect on circadian organization; a small deviation in Q_{10} from 1.0 is sufficient to produce large changes in tau. For example, CRs of melatonin secretion from

1. To whom all correspondence should be addressed.

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the lizard pineal gland have a Q_{10} of 1.135 in vitro; this represents a 5-h shortening of tau when temperature is increased from 25 to 37°C (Menaker and Wisner, 1983).

It has been difficult to test the principle of temperature compensation in endotherms because they homeostatically defend brain and body temperatures (T_{br}, T_b) within a narrow range relative to the range of ambient temperatures (T_as) they experience, thus making direct manipulations of the pacemaker difficult. Evidence for temperature compensation of circadian pacemakers in homeothermic mammals has been reported for a few rodent species, but the extreme environmental manipulations used to lower core T_b and the failure to monitor T_{br} make those results difficult to interpret (Gibbs, 1981, 1983; Rawson, 1960). In contrast to obligate homeotherms, hibernators and animals that express shallow daily torpor experience substantial decreases in T_b and T_{br} that are associated with substantial changes in tau. For example, pocket mice arouse from torpor every 1-3 days during the hibernation season (Bartholemew and Cade, 1957; Lindberg and Hayden, 1974). Arousals occur at intervals that are multiples of nearly 24 h in animals housed in constant darkness; by decreasing T_a from 21 to 10°C, tau of arousals was shortened by > 1.0 h (Lindberg et al., 1971). It would be inappropriate to calculate a Q_{10} for tau in this context, however, because T_{br} was not measured. Nevertheless, tau is affected by changes in T_a normally experienced by pocket mice in the wild.

It is possible that temperature-induced changes in tau observed in behavioral CRs reflect a direct action on the circadian pacemaker. In mammals, the hypothalamic suprachiasmatic nucleus (SCN) contains a circadian pacemaker that, in homeotherms, experiences small changes in temperature (e.g., 2-3°C). Although many chronobiologists might assume that changes in SCN temperature of only a few degrees are insufficient to alter SCN timekeeping functions, this assumption has never been tested. A basic tenet of CRs is that changes in tau of an overt rhythm reflect similar changes in the period of the pacemaker driving that rhythm (Pittendrigh, 1974; Pittendrigh and Daan, 1976b). Pocket mice maintain T_{br} slightly above T_a during torpor; therefore, a bout of torpor can be associated with change in SCN temperature of ≥10°C. A temperature change of this magnitude may be sufficient to alter pacemaker period in the SCN and produce a corresponding change in behavioral rhythms.

The aim of the present study is to assess the extent of temperature compensation in the SCN. The oscillatory properties of the SCN persist for several days in vitro; CRs in spontaneous neuronal activity have been recorded for 3 days from brain slices containing the SCN (Ding et al., 1994; Prosser and Gillette, 1989; Shibata and Moore, 1993a). Neuronal activity is a widely used index of CRs in vitro because it is an extremely stable and reproducible rhythm (cf. Gillette, 1991). In this study, the SCN from a hibernator (i.e., ground squirrel) and from an obligate homeotherm (i.e., rat) were compared because temperature compensation may be more robust in animals that experience large changes in T_{br} (> 30°C) than it is in homeotherms. By contrast, the relatively small changes in T_{br} that rats experience may have relaxed selection pressure for temperature compensation.

METHODS

Housing Conditions

Male golden-mantled ground squirrels (*Spermophilus lateralis*) were trapped in mid-September 1992 near Stampede Reservoir in the Sierra Nevada Mountains at approximately 6000 ft above sea level. Body mass (150-200 g) indicated that they were juveniles; adults and yearlings typically weigh > 300 g in late summer or early fall (Kenagy, 1986). In the laboratory, squirrels were fed chow (Purina no. 5012) and sunflower seeds and were provided with cotton batting for nesting material. Squirrels and adult male Wistar rats (Simonsen) were housed in the laboratory in a 12:12 light:dark (LD) cycle (fluorescent lights on at 0800 h Pacific Standard Time); squirrels and rats were housed at T_as of 6 and 22°C, respectively. Squirrels were moved to a warm room (T_a = 22°C) with the same LD cycle 16 h prior to tissue slice preparation to ensure that they were normothermic when brain slices were prepared. Food and water were available ad libitum.

Brain Slice Preparation and Maintenance

All brain slices were prepared within 1 h after lights on in the animal room. After rapid decapitation, the optic tracts were severed and the brains were quickly removed. The hypothalamic region was then blocked and 500-μm coronal slices were prepared on a tissue chopper (Sorvall). Tissue slices containing the paired SCN, optic chiasm, and minimal surrounding tissue were incubated in a Hatton-style brain slice chamber (Hatton et al., 1980) warmed to 37 or 25°C and gassed continuously with 95% O_2/5% CO_2. Brain slices were

allowed at least 1 h to equilibrate before electrical recording.

Calibration of the temperature in the tissue slice chamber was accomplished by inserting a thin wire thermocouple, which was connected to a digital thermometer (Sensortek), into a tissue slice dorsal to the SCN. The slice chamber was then closed around the thermocouple, and water bath temperature was adjusted to maintain the brain slice at precisely 37 or 25°C. Tissue slices were continuously perifused at 35 ml/h with Earle's Balanced Salt Solution (Sigma) supplemented with 24.6 mM glucose and 26.2 mM sodium bicarbonate (pH 7.4). For tissue slices maintained longer than 36 h, the medium was supplemented with an antibiotic (0.05% gentamicin). Under these conditions, the SCN remains viable and exhibits robust CRs in spontaneous neuronal activity for at least 60 h (Gillette, 1991).

Electrophysiological Recording

Extracellular recordings from single cells were made using glass microelectrodes filled with 3 M NaCl. The electrode was lowered into the SCN with a hydraulic microdrive until action potentials were observed. Spikes with amplitudes at least twice that of background noise were observed for 2 min to verify steady firing patterns; only cells with stable firing rates and amplitudes were recorded. After the firing rate of a cell was recorded for 5 min, the electrode was advanced until another cell was encountered. The repetition of this process over 8 to 36 h constituted a recording trial. Each electrode track in the SCN was placed randomly, and cells were recorded throughout the cross-sectional extent of each SCN. All data were stored on computer for subsequent analysis (DataWave, formerly BrainWave).

Data Analysis

The average firing rate of each cell was determined by calculating the reciprocal of its mean interspike interval. Mean (± *SE*) hourly firing rates were calculated by a running average with a 2-h window and 1-h lag (Gillette, 1991; Prosser and Gillette, 1989). Peak neuronal firing rate was defined as the single highest hourly value obtained on the day of recording. Because there occasionally was no clear daily trough in firing rate, trough values were defined as the mean firing rates of all cells recorded during the 3-h interval centered between successive daily peaks. Rhythm amplitude is the difference between peak and trough values on Day 1.

CR phase was defined as the time of peak firing rate relative to the time of lights on in the donor colony; tau was defined as the interval between consecutive daily peaks. Hour 0 was defined as the time of lights on in the donor colony on Day 1 and the projected time of lights on for Days 2 and 3 in vitro. Time of peak firing rate has been used extensively as a reliable index of circadian pacemaker phase (cf. Gillette, 1991). The effects of temperature on tau, firing rate, and rhythm amplitude were assessed by calculating Q_{10} values for these rates at 37 and 25°C. Differences among groups in peak and trough firing rates were determined by two-way analysis of variance or *t* test where appropriate.

The effects of temperature on the temporal distribution of cells with high discharge rates was investigated with data from Day 2. Mean firing rates were calculated separately for rats and squirrels at 37 and 25°C. The time of day when a cell was recorded was determined only for those cells that had firing rates greater than their group mean. Differences between groups in the temporal distribution of these cells was assessed by Bartlett's chi-square test for homogeneity of variance; the test for significant differences between two variances (i.e., F_s variance ratios) was used for all subsequent pairwise tests.

RESULTS

Neuronal Firing Rate

Squirrels. Cell recordings were made from 33 squirrels, 1 animal per recording session; 453 and 401 cells were recorded at 37 and 25°C, respectively. Firing rates were significantly lower at 25°C than they were at 37°C on all 3 days, $F(1, 140) = 10.02$, $p < .001$. There were also significant decreases in peak firing rates on successive days at 37 and 25°C, $F(2, 140) = 8.41$, $p < .001$ (Table 1). The trough firing rate was slightly, but significantly, higher at 25°C than it was at 37°C on Day 1, $t = 5.88$, $p < .05$. Peak firing rates were highly temperature compensated; Q_{10} values ranged from 1.14 to 1.31 (Table 1).

Rats. Cells were recorded from rat SCN over 32 trials; 305 and 565 cells were recorded at 37 and 25°C, respectively. Peak firing rates did not decline signifi-

Table 1. Temperature effects on peak and trough firing rates for rats and squirrels.

Animal	*37°C*	*25°C*	Q_{10}
Squirrel			
Peak Day 1	4.30 ± 0.44 (30)	3.10 ± 0.42 (21)*	1.31
Peak Day 2	3.80 ± 0.35 (35)	2.74 ± 0.28 (33)*	1.31
Peak Day 3	2.47 ± 0.30 (22)	2.12 ± 0.17 (35)*	1.14
Trough Day 1	1.40 ± 0.04 (18)	1.51 ± 0.06 (18)*	0.94
Rat			
Peak Day 1	7.12 ± 0.45 (23)	2.14 ± 0.26 (32)*	2.72
Peak Day 2	7.50 ± 0.50 (18)	1.98 ± 0.23 (39)*	3.00
Peak Day 3	5.28 ± 0.51 (19)	n.a.[a]	—
Trough Day 1	2.10 ± 0.33 (22)	1.57 ± 0.28 (35)*	1.27

NOTE: Firing rates represent mean (± *SE*) values of individual neurons combined from two to six trials. Numbers of cells are in parentheses.
a. Data not available because a clear peak was not observed in this group.
*$p < .05$ compared to same-day value at 37°C.

cantly on successive days at 37°C, $F(2, 57) = 2.82$, $p > .05$. Exposure to 25°C suppressed firing rates substantially; peak firing rates were decreased significantly by 70% on Day 1 ($t = 47.66$, $p < .001$) and by 74% on Day 2 ($t = 44.71$, $p < .001$) (Table 1). However, trough firing rates decreased by only 25%, $t = 6.25$, $p < .001$ (Table 1). The magnitude of these differences is reflected by the Q_{10} values for peak firing rate, which ranged from 2.72 to 3.00; these values were more than double those obtained from squirrels (Table 1).

Circadian Organization

Squirrels. CRs in neuronal activity were readily detected in ground squirrel SCN at 37 and 25°C. Firing rates on Day 1 peaked during midsubjective day and remained low throughout subjective night (Figure 1). The mean (± *SE*) time of peak firing rates occurred 7, 6, and 5 h after the projected time of lights on in the donor colony on Days 1, 2, and 3, respectively, at 37°C (Figure 1).

The timing of peak neuronal activity was affected substantially by temperature; daily peak firing rates occurred 2, 3, and 5 h earlier at 25°C than they did at 37°C on Days 1, 2, and 3, respectively (Figure 1). Recordings from individual trials reveal that there was no variability in peak time on all 3 days at 37°C and on Days 1 and 2 at 25°C (Table 2 and Figure 2). The lack of variability in timing of peak firing rates precludes statistical analysis of these data; intertrial variability of less than 1 h may occur, however, and would not be detected using a 1-h lag in the running average.

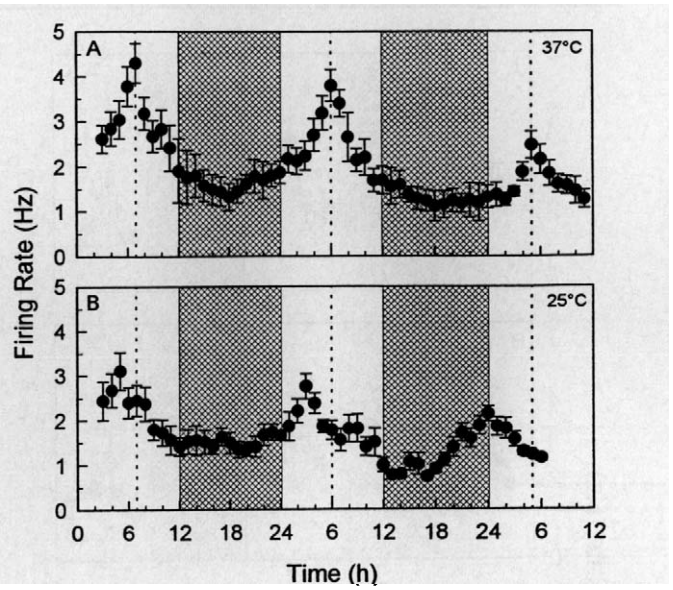

Figure 1. Mean (± *SE*) hourly firing rates of squirrel suprachiasmatic nucleus neurons. Each hourly value represents the mean of three to six separate trials. All tissue slices were prepared between 0 and 1 h on Day 1. Shaded areas represent projected dark phases of the light:dark (LD) cycle in the donor colony (LD 12:12, lights on at 0 h). Vertical dashed lines represent time of peak firing rates at 37°C to ease comparison to 25°C. Tissue slices were maintained at (A) 37°C or (B) 25°C.

Table 2. Temperature effects on circadian timing of peak firing rates in rats and squirrels.

Animal	*37°C*	*25°C*
Squirrel		
Day 1	7.0 ± 0.0 (3)	5.0 ± 0.0 (6)
Day 2	6.0 ± 0.0 (3)	3.0 ± 0.0 (5)
Day 3	5.0 ± 0.0 (3)	0.0 ± 0.5 (3)
Rat		
Day 1	6.0 ± 0.0 (2)	10.0 ± 0.5 (4)
Day 2	6.0 ± 0.0 (2)	11.3 ± 1.3 (4)[a]
Day 3	6.0 ± 0.0 (3)	[b] (6)

NOTE: Values are the hour of day after the projected time of lights on (0 h) for that day. No statistical comparisons could be conducted because there was no variability in the time of peak at 37°C on all 3 days for squirrels and rats. Numbers of trials are in parentheses.
a. Based only on trials in which peaks could be determined.
b. No clear peak time could be determined for that day.

Tau was shortened by exposure to low temperature. The interval between all peaks at 37°C was 23.0 h; this interval shortened to 22.0 and 21.0 h on Days 1-2 and 2-3, respectively, at 25°C (Figures 1B and 2C,D). The mean tau at 25°C was 21.5 h with a mean Q_{10} of 0.945. The Q_{10}s associated with period changes on Days 1-2 and 2-3 are 0.964 and 0.927, respectively. Exposure of tissue slices to 25°C reduced rhythm amplitude by

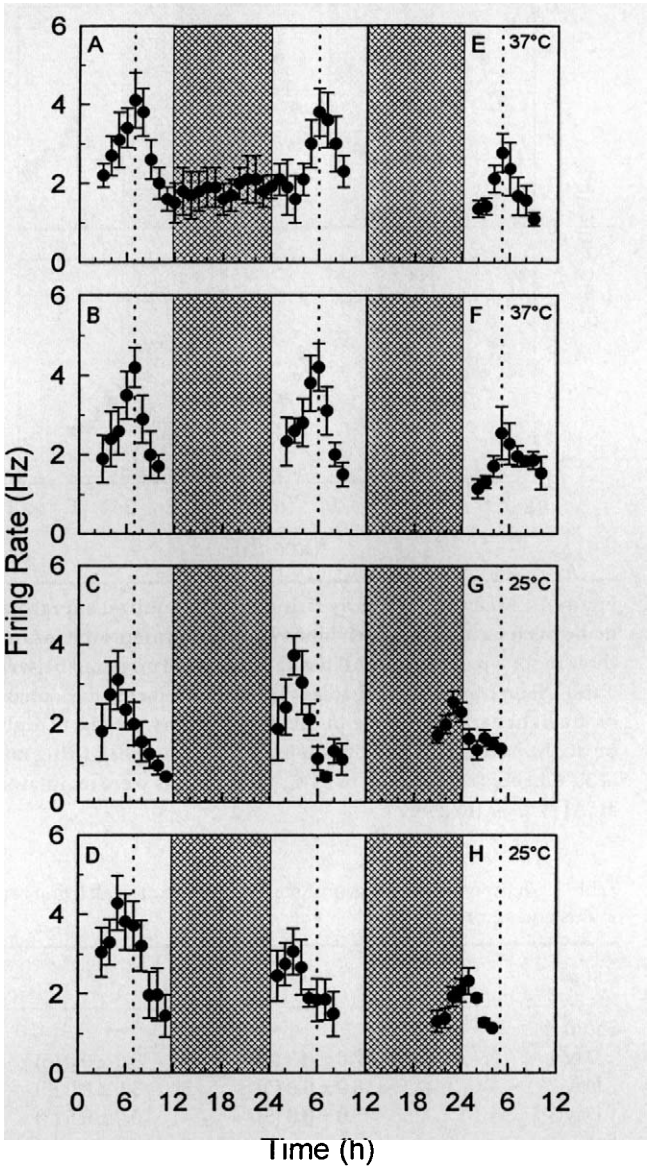

Figure 2. Representative individual trials recorded from squirrel suprachiasmatic nucleus slices. Data are plotted as mean (± *SE*) hourly firing rates. Each panel is a recording from a single tissue slice during Days 1 and 2 (A-D) or Day 3 (E-H) in vitro at 37°C (A,B,E,F) or 25°C (C,D,G,H). Shaded areas and vertical dashed lines represent the same as they do in Figure 1.

45% of its normal value on Day 1 (2.90 vs. 1.59 Hz [Figure 1]).

Rats. Circadian neuronal rhythms were robust and stable in the rat SCN at 37°C; peak firing rates occurred 6 h after the projected time of lights on on all 3 days (Figure 3A). By contrast, on Days 1 and 2, peak firing rates occurred 4.0 and 5.3 h later, respectively, at 25°C than they did at 37°C (Figure 3B and Table 2). Due to

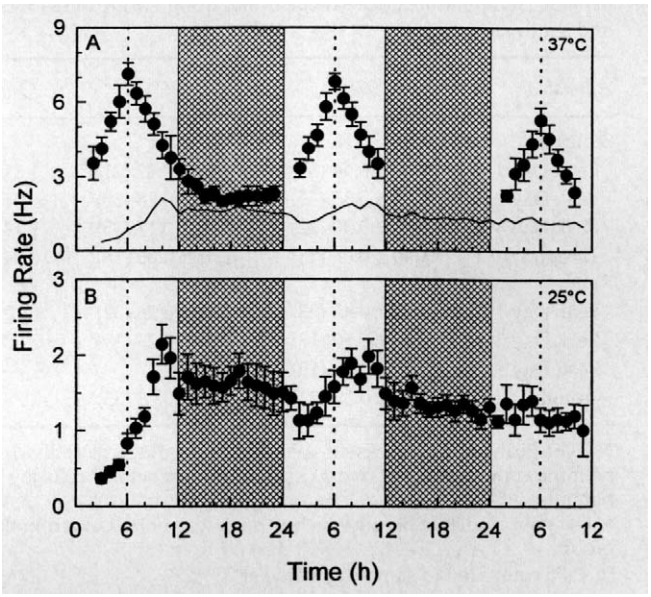

Figure 3. Mean (± *SE*) hourly firing rates of rat suprachiasmatic nucleus neurons. Each hourly value represents the mean of two to six separate trials. All tissue slices were prepared between 0 and 1 h on Day 1. Data in panel B are replotted as solid line in panel A to facilitate comparison to data obtained at 37°C. Shaded areas and vertical dashed lines represent the same as they do in Figure 1.

the lack of variability in peak times of individual trials at 37°C, temperature effects on timing of peak firing rates could not be statistically analyzed.

In contrast to the case with squirrels, clear peaks were not always present in individual trials at 25°C (Figure 4); when present, the timing of peaks on Days 1 and 2 at 25°C was highly variable (Table 2). The mean (± *SE*) times of peak firing rate were 10.0 ± 0.5 and 11.3 ± 1.3 h on Days 1 and 2, respectively, and ranged from 9 to 11 h (n = 5) and from 8 to 15 h (n = 6) after the projected time of lights on in individual trials on Days 1 and 2, respectively (Figure 4). Tau was 24.0 h at 37°C, but the lack of consistent and discrete peaks on Days 2 and 3 prevented reliable estimation of tau at 25°C.

The amplitude of the circadian neuronal rhythm was severely dampened at 25°C on Days 1 and 2, and clear peaks were never observed on Day 3 in group or individual data (Figures 3,4). Rhythm amplitude was decreased by 89% of its 37°C value (5.02 vs. 0.57 Hz [Figure 3]); this is nearly twice as great a reduction in amplitude than was observed in the squirrel SCN.

Detection of Single Units

The effect of temperature on the ability to detect stable single units was tested using data collected on Day 1; the detection of stable single units was affected

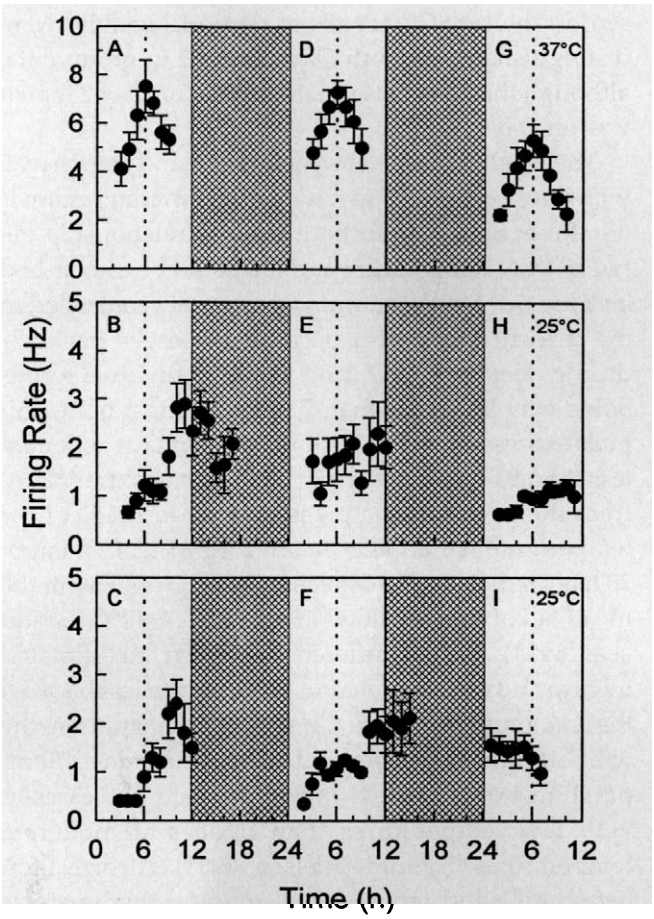

Figure 4. Representative individual trials recorded from rat suprachiasmatic nucleus slices. Data are plotted as mean (± *SE*) hourly firing rates. Each panel represents an individual trial in a different animal recorded on Day 1 (A-C), Day 2 (D-F), or Day 3 (G-I) at 37°C (A,D,G) or 25°C (B,C,E,F,H,I). Shaded areas and vertical dashed lines represent the same as they do in Figure 1.

significantly by temperature ($F[1, 95] = 21.79$, $p < .001$) and species ($F[1, 95] = 5.63$, $p < .05$). The number of cells recorded per hour was not significantly different between rats and squirrels at 37°C ($t = 0.47$, $p > .05$) but was different at 25°C ($t = 16.43$, $p < .001$) (Figure 5). The number of cells recorded per hour in rat SCN slices was substantially lower at 25°C than it was at 37°C ($t = 22.81$, $p < .001$), but this effect was less robust in squirrel SCN slices ($t = 6.04$, $p < 0.001$) (Figure 5); these represent 37 and 11% reductions in the number of cells recorded per hour for rats and squirrels, respectively (Figure 5).

Temporal Distribution of Individual Neurons

Effects of temperature on the temporal distribution of single unit firing rate were analyzed in cells that had

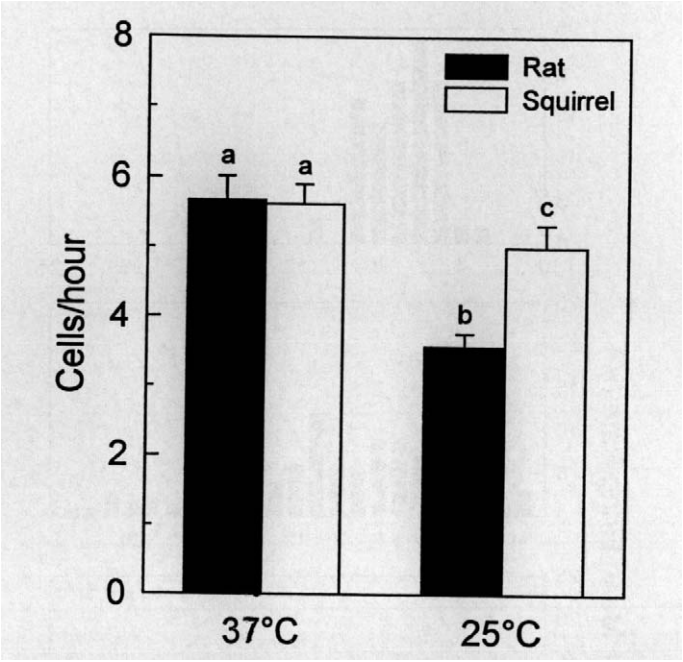

Figure 5. Mean (± *SE*) number of cells recorded per hour. Data are combined from all trials recorded over the first 24 h in vitro. Bars with different letters are significantly different from each other ($p < .05$).

firing rates greater than the daily mean for their group (Figure 6). The normal temporal distribution of high firing rates was disrupted by low temperature; variances were not homogeneous among these four groups, $\chi^2 = 18.89$, $p < .001$. This effect was significant for rats ($F_s(8, 24) = 9.32$, $p < .01$) but not for squirrels ($F_s(8, 12) = 1.72$, $p > .05$). Elevated firing rates were observed over a range of 8 and 12 h in rats and squirrels, respectively, at 37°C. By contrast, high firing cells were found at all hours of the day in rats but only over a range of 8 h in squirrels at 25°C (Figure 6). Mean firing rates were 5.12 Hz ($n = 42$) and 2.23 Hz ($n = 70$) at 37°C and were 1.52 Hz ($n = 101$) and 1.79 Hz ($n = 69$) at 25°C in rats and squirrels, respectively.

DISCUSSION

Temperature Effects on Circadian Organization

Temperature compensation is a fundamental and defining property of CRs that has been demonstrated in several nonmammalian species. The present study is the first direct test of this principle in a mammalian circadian pacemaker, the SCN. The tau of SCN neuronal CRs in ground squirrels had a Q_{10} of 0.927 between

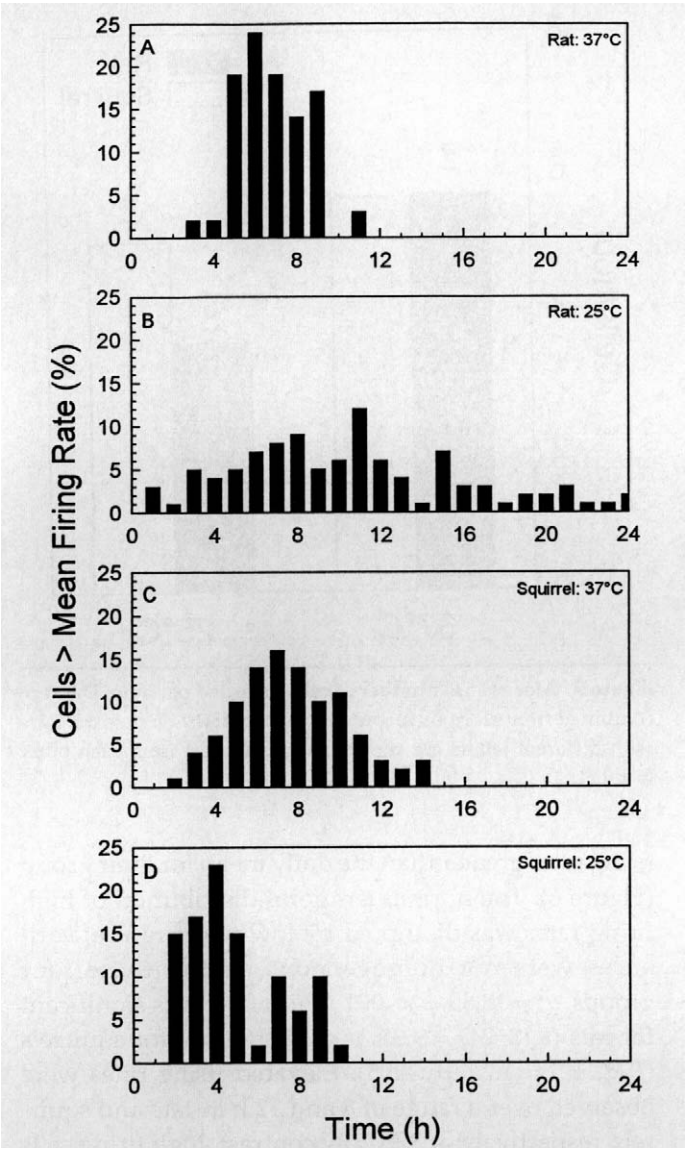

Figure 6. Percentage of cells with firing rates greater than the 24-h daily mean recorded during each hour of Day 2. Cells were recorded from rat suprachiasmatic nucleus (SCN) at (A) 37°C or (B) 25°C and from squirrel SCN at (C) 37°C or (D) 25°C. The variance in the distribution of neurons in panel B was significantly different from the other groups ($p < .001$).

temperatures of 37 and 25°C. This Q_{10} is within the limits normally accepted as evidence for temperature compensation (Pittendrigh, 1974). Because most biological processes have Q_{10}s of from 2 to 3, a Q_{10} for tau that varies little from 1.0 is considered strong evidence that pacemaker frequency is well conserved across a broad temperature range. In contrast to the case with squirrels, circadian SCN neuronal rhythms in rats were severely dampened and disrupted at 25°C. Although CR amplitude was greatly diminished at 25°C, one cannot say definitively that CRs were completely absent in the rat SCN. Peaks in neuronal activity were distinguishable on both Days 1 and 2 in group data, although the peak was much broader on Day 2 than it was on Day 1.

The results obtained in this in vitro study agree well with those obtained in vivo in hibernating animals (Grahn et al., 1994). In both cases, a relationship between SCN temperature and tau could be established because SCN temperature was precisely controlled in the present study and because it closely tracks T_b during deep torpor. During winter, individual torpor bouts may last more than 7 days; within a bout, animals express circadian T_b rhythms with taus generally less than 24 h (Grahn et al., 1994). If one extrapolates from the results of the present study to predict how temperature would alter tau at a T_{br} of 12°C, a tau of 20 h is predicted. This agrees reasonably well with the mean tau of 22 h obtained in vivo at T_b = 12°C (Grahn et al., 1994). The real difference between tau estimated in vitro and tau estimated in vivo may be less because the Q_{10} measure is not linear and depends on the temperature range over which it is determined. Temperature exerts far less impact on rate processes at very low temperatures than it does at moderate temperatures (Schmidt-Nielsen, 1983). A correlation between T_a and tau of wheel-running rhythms was found in this species (Lee et al., 1990); decreases in T_a were associated with longer taus. As in the case of the pocket mouse discussed in the Introduction, a comparison between the study by Lee et al. (1990) and the present data cannot be made, however, because that study did not assess the relationship between the temperature of the SCN and tau.

The Q_{10}s obtained for ground squirrel SCN are similar to those estimated for chick pinealocytes. The chick pineal is the only other preparation from an endotherm in which temperature compensation has been tested by directly manipulating pacemaker temperature. Although the ground squirrel SCN appears to be better temperature compensated than the chick pineal (Q_{10} = 0.945 vs. ≈ 0.84) (Barrett and Takahashi, 1995; Zatz et al., 1994), it is important to note that the period shortens in both pacemakers as temperature declines. It is unclear, however, why the squirrel SCN is less temperature sensitive than are chick pinealocytes. Hibernators undergo acclimatization prior to winter that affects electrical and neurochemical functions in the brain (Heller, 1979; Pakhotin et al., 1993); neurophysiological function in the SCN may be altered in winter-adapted squirrels compared to that in summer-condition animals. Thus it would be inappropriate to

overgeneralize data obtained from hibernating squirrels to other mammals.

Because the SCN experiences a sudden temperature change when it is transferred into a 25°C tissue chamber during slice preparation, one may argue that the present results based on three circadian cycles are transient phase or period changes that do not represent stable changes in tau. Although tau generally is estimated from more than three circadian cycles in whole organisms, this is not always possible in isolated pacemaker preparations (cf. Zatz et al., 1994). It is unlikely that the present data reflect transient phase shifts because such transients are characterized by large phase shifts in the first cycle after a phase-shifting stimulus followed by comparatively smaller phase shifts prior to a stable free run (Pittendrigh, 1974); this trend was not observed in the present study. Further, transient phase shifts never have been observed in any of the numerous studies that have employed the SCN brain slice preparation, even when a final phase advance of 6 h is achieved (Ding et al., 1994; Gillette, 1991; Prosser and Gillette, 1989; Shibata and Moore, 1993b).

Temperature Effects on Neuronal Firing Rate

Low temperature disrupted CRs by affecting neuronal activity in three different ways. Compared to the case in squirrel SCN, in rat SCN, (1) peak firing rate was much more sensitive to temperature, (2) fewer single units were active, and (3) variability in the timing of peak firing rates increased substantially at 25°C. The decreased number of active single units in the rat SCN at 25°C is most likely due to the failure of some neurons to discharge below this temperature. Most SCN neurons fail to generate action potentials below 24.4°C (Miller et al., 1994). Because Q_{10}s for firing rate in rat SCN neurons range from 2 to 3 (Table 1; Miller et al., 1994), quiescence of some neurons is expected at 25°C. The differential effects of temperature on individual rat neurons do not, however, satisfactorily explain the disruption of CRs at low temperature because temperature sensitivity is greater in individual squirrel neurons than it is in individual rat SCN neurons (Miller et al., 1994) and neuronal CRs remain intact in squirrel SCN at 25°C. Thus temperature may affect CRs via some mechanism independent of its effects on neuronal discharge.

Increased variability in the temporal distribution of rat SCN neurons at low temperature suggests a mechanism by which temperature disrupts CRs. The time of day that these cells were recorded was investigated because daily peaks in neuronal activity generally are composed of a high proportion of cells firing at or near their maximum rate during a relatively short interval (i.e., 2 to 4 h). Thus we investigated whether the lack of clear peaks at this temperature was due to a change in the distribution of cells with relatively high firing rates across the day. The issue was investigated in two ways; a cell was considered to have a high firing rate if its discharge rate exceeded the daily mean firing rate for its group or if its firing rate was > 4 Hz. Because both methods yielded similar results, only one has been presented here (Figure 6). Despite similar daily mean firing rates, the distribution of cells with elevated discharge rates was well preserved in squirrels but not in rats at 25°C. We suggest that rat SCN neurons may have become temporally desynchronized from one another and that such desynchronization contributed to the loss of circadian organization at 25°C. Some individual SCN neurons exhibit CRs in neuronal activity in cultures that have periods ranging from ≈ 21 to 26 h (Welsh et al., 1995). Assuming such "pacemaker" cells exist in the slice, low temperature may phase shift or alter the periods of these neurons. In either case, lowering the temperature would desynchronize CRs of individual neurons; the squirrel SCN may be better adapted to maintaining circadian phase synchronization among neurons than is the rat SCN at low temperature.

Temperature compensation of the SCN pacemaker does not require that individual neurons be temperature compensated. In fact, firing rates for individual warm-sensitive SCN neurons in tissue slices from winter-adapted ground squirrels have Q_{10}s > 3.0 (Miller et al., 1994). This does not preclude the possibility, however, that other neurons may be temperature compensated because only a relatively small number of SCN neurons has ever been tested (Miller et al., 1994). Alternatively, temperature compensation of tau may be a property of neuronal interactions. The interaction of warm- and cold-sensitive neurons may help maintain pacemaker frequency during the rapid and broad changes in T_{br} (> 30°C) that occur during entrance and arousal from torpor. The observation that cold-sensitive SCN neurons are found in greater numbers in hibernating squirrels than in normothermic ones or rats (Miller et al., 1994) supports this conjecture. It is unlikely, however, that interactions between cold- and warm-sensitive neurons could be

responsible for CRs below ≈ 17°C because neuronal discharge has not been observed in rat or squirrel SCN neurons below this temperature in recordings made with extracellular electrodes. Because action potential amplitude decreases as temperature declines (Krilowicz et al., 1989), other recording techniques that are more sensitive may reveal action potentials below 17°C. Nevertheless, relatively high metabolic activity persists in the SCN and a few other hypothalamic structures, but not other brain regions, during deep hibernation (Kilduff et al., 1982). The SCN may be able to maintain timekeeping or other functions independent of action potential generation during hibernation.

Low temperature suppressed peak firing rates in rats far more than it did in squirrels. In this regard, the SCN is similar to other brain regions. Thermosensitive neurons in the preoptic area of the hypothalamus continue to discharge spontaneously at 10°C in winter-adapted golden hamsters but cease activity below 28°C in guinea pigs in vivo (Wünnenberg et al., 1976). Hippocampal slices from cold-acclimated hamsters have lower temperature thresholds for evoking population spikes than do warm-acclimated hamsters (Thomas et al., 1986). Spontaneous activity of single units in the medial septal nucleus taken from hibernating ground squirrels is twice that observed in waking ground squirrels and guinea pigs in vitro (Belousov et al., 1990); tissue slices from hibernators also tolerate longer intervals at low temperatures (2 to 4°C) better than do those from nonhibernators (Pakhotin et al., 1990). All of these brain regions have been implicated in the neural control of hibernation. It is difficult, however, to determine whether enhanced cold tolerance suggests a functional role for these brain regions in hibernation or simply protects the brain from thermal damage during deep torpor.

CONCLUSIONS

Asserting that a circadian pacemaker is temperature compensated implies that temperature has little effect on pacemaker period. Q_{10} values only slightly different from 1.0 can, however, alter period and phase considerably; a Q_{10} of 0.927 was associated with a shortening of tau by 2 h that resulted in a 5-h phase change in the squirrel SCN after only 48 h at low temperature. Although temperature compensation is a critical requirement for accurate biological timekeeping, it is important to note that a change in Q_{10} of only 0.1 has substantial effects on circadian organization. The commonly accepted definition of temperature compensation for circadian pacemakers that tau have a Q_{10} "near 1.0" has been met and documented for circadian pacemakers in cyanobacteria (Kondo et al., 1993), *Acetabularia* (Berger et al., 1992), *Gonyaulax* (Hastings and Sweeney, 1957), *Neurospora* (Mattern et al., 1982), *Drosophila* (Huang et al., 1995; Pittendrigh, 1954), *Aplysia* (Benson and Jacklet, 1977), and lizard and chick pinealocytes (Barrett and Takahashi, 1995; Menaker and Wisner, 1983; Zatz et al., 1994). Based on the Q_{10}s reported in these studies, our calculations show that a temperature change of only 3°C, within the thermal range normally experienced by these organisms, produces a *minimum* change in tau of 1.0 h in all of these cases except *Drosophila* and *Aplysia*. For example, the tau of pineal melatonin secretion from chick pinealocytes has Q_{10}s of 0.83 (Barrett and Takahashi, 1995) and 0.84 (Zatz et al., 1994) between ≈ 37°C and 40°C; this represents a change in tau of 1.3 h over just a 3°C temperature range. Even by conservative standards, this represents a substantial change in tau.

These results raise the interesting possibility that normal daily changes in T_{br} may influence overall circadian organization in organisms despite the fact that their pacemakers are temperature compensated. Daily variations of cortical and preoptic area temperature have an amplitude of ≈ 2.0°C in rats housed without access to running wheels (Abrams and Hammel, 1965; Franken et al., 1992). Wheel running, feeding, and drinking produce rapid increases in preoptic area temperature of 1.0 to 2.5°C that often are sustained for several hours (Abrams and Hammel, 1965). Thus brains of rats with access to running wheels experience daily and short-term temperature changes of ≈ 2.5°C (Abrams and Hammel, 1965). Based on Q_{10}s obtained in the present study, a change in T_{br} of 2.5°C would be sufficient to produce significant changes in tau. Whether short-term changes in T_{br} influence circadian organization is presently unknown. Nor is it known what degree of temperature sensitivity is seen in the rat SCN pacemaker in the range of temperatures that it normally experiences. It is interesting to speculate, however, whether the increased T_{br} associated with locomotor activity is part of the mechanism by which activity feedback phase shifts the pacemaker, particularly in light of the finding that high activity levels are required to produce phase shifts.

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