Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold

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Robinson, Edward L., and Charles A. Fuller. Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1397–R1407, 1999.—Whole body heat production (HP) and heat loss (HL) were examined to determine if the free-running circadian rhythm in body temperature (T_b) results from coordinated changes in HP and HL rhythms in thermoneutrality (27°C) as well as mild cold (17°C). Squirrel monkey metabolism (n = 6) was monitored by both indirect and direct calorimetry, with telemetered measurement of T_b and activity. Feeding was also measured. Rhythms of HP, HL, and conductance were tightly coupled with the circadian T_b rhythm at both ambient temperatures (T_A). At 17°C, increased HP compensated for higher HL at all phases of the T_b rhythm, resulting in only minor changes to T_b. Parallel compensatory changes of HP and HL were seen at all rhythm phases at both T_A. Similar time courses of T_b, HP, and HL in their respective rhythms and the relative stability of T_b during both active and rest periods suggest action of the circadian timing system on T_b set point.

body temperature; direct calorimetry; indirect calorimetry; metabolism; heat production; heat loss; nonhuman primates; conductance

PRIMATES, INCLUDING HUMANS, typically possess a robust circadian rhythm of body temperature (T_b). In the absence of external time cues, or zeitgebers, T_b rises and falls in a daily, or circadian, rhythm with a period of ~24 h. Changes in thermoregulatory effectors that contribute to this rhythm have been described and include heat production (HP), or metabolic rate (4, 17, 18, 21, 28, 37); heat loss (HL), including vascular changes (19, 42); body temperature changes (18, 48), and thermal conductance (C); and whole-body HL rhythms (4, 5, 37).

In thermoneutrality, HP increases near the beginning of the active period (a) and increased HL is phase delayed, resulting in net heat gain and producing a large increase in T_b. Similarly, decreased HP, followed by decreased HL, produces a net HL from the body around the beginning of the rest period (p), resulting in a large decrease in T_b (4, 5, 37). Combined, these changes in heat content produce the circadian rhythm in T_b.

Rhythms in both colonic and hypothalamic T_b have been shown for squirrel monkeys (14, and T_b rhythms have been shown in a variety of other homeotherms (for a recent review, see Ref. 36). However, relatively few studies have examined thermoregulatory rhythms at ambient temperatures (T_A) outside the thermoneutral zone. For example, in primates, changes in brain or T_b means and amplitudes with T_A have been shown in pig-tailed macaques (45) and squirrel monkeys (14, 15). Changes in the period of the T_b rhythm due to T_A were also observed in the pig-tailed macaques.

Although changes in HP and thermal C rhythms have been demonstrated outside of thermoneutrality, the interrelationships of the whole body HP, HL, and T_b rhythms have not, to our knowledge, been examined in the cold. This study was conducted to test the hypothesis that the free-running circadian T_b rhythm results from coordinated rhythms of HP and HL in mild cold as well as thermoneutrality. In addition, rhythm properties, representing output of the circadian timing system (CTS), might be expected to change due to altered T_b homeostasis in the cold.

METHODS

Protocol. Six adult male squirrel monkeys (Saimiri sciureus) with an average body mass of 1.05 kg (0.90–1.17 kg) were studied. Animals were conditioned to constant light (LL) for at least 7 days before the experiment. T_A during acclimation was 27 ± 3°C. Ambient light intensity from an overhead cool white fluorescent fixture averaged 200 lx in the cage. The animal was provided with a pelleted diet (190-mg banana pellets, no. F0035; Bioserv) and water ad libitum.

At the beginning of an experiment, the animal was weighed and placed unrestrained into the calorimeter for a 24-h acclimation period. Thermoneutrality studies were conducted at 27 ± 1°C (43). For cold studies, T_A was 17 ± 1°C, below thermal neutrality and presenting a mild cold stress previously shown to increase resting HP by ~60% (43). Light in the calorimeter was from a fiber-optic light with a quartz halogen source (Dolan-Jenner Fiber Lite 180) and averaged 200 lx. To reduce the effects of transients (16), we discarded data from the initial 24-h acclimation period, after which 6 days of data were collected at 10-min intervals by a microcomputer data acquisition system (Dataquest III, Data Sciences). The calorimeter was opened for waste removal and visual inspection of the monkey at ~36-h intervals. Data for the subsequent hour were discarded. Additional short gaps in the data resulted from loss of the telemetry signal and from sampling of inlet air.

Measurements. T_b and activity were measured as previously described (37), using a biotelemetry transmitter implanted in the peritoneum. HP was determined by measuring oxygen consumption. In this study, CO2 production was not measured, and an average respiratory quotient of 0.79 was assumed based on previous measurements (5 monkeys over 6 days). Continuous measurements of relative humidity were used to determine evaporative HL (EHL) and to correct gas measurements to fraction in dry air. Dry HL (DHL) was measured using direct calorimetry. Total HL was calculated as the sum of DHL and EHL. Total body thermal C was
calculated at 10-min intervals as $C = \frac{HP}{(T_b - T_A)}$ using the formula of Bradley and Deavers (9) for total whole body $C$.

Food and water were available ad libitum. Animals were supplied with a tap switch that operated an automated feeder (Gerbrands G5100). Feeding was monitored continuously.

Data analysis. Average waveforms were determined by eduction of the data for each variable based on the rhythm period ($\tau$) for each animal. This procedure averages all data for a variable at the same rhythm phase, resulting in an average waveform (30). The reference phase for an animal’s rhythms, nominally 0° circadian phase (CP), was defined as the ascending median crossing for $T_b$. Average waveforms were calculated after weighted interpolation to 144 points per cycle (2.5 per point) for each animal. Rhythm means and acrophases ($\phi$), or estimated phase angles of rhythm peaks, were determined from original data using the cosinor method (22, 23). This method also estimates rhythm amplitude from the fitted cosine function. Differences in rhythm means, $\phi$, and amplitudes between $T_A$ were tested by multivariate ANOVA (MANOVA) (SPSS). Differences between $T_A$s that were significantly different from zero were accepted based on Pillai’s trace statistic for multiple comparisons of $T_b$, HP, HL, feeding, and $C$, and on univariate $F$ tests for individual variables, assuming critical probabilities of 0.05. Because activity measurements were sensitive to the quality of radio reception and, in some cases, were made with different transmitters at the two $T_A$, quantitative comparisons of activity were not possible.

For each animal, onsets of $a$ and $r$ were determined from average waveform eductions for each variable as the ascending and descending median crossings, respectively. Phase angles for $a$ and $r$ onsets were standardized to degrees of the individual animal’s $\tau$, equal to a 360° cycle, relative to $T_b$ onset, defined as zero degrees CP at each $T_A$. Differences in onset of $a$ and $r$, in degrees CP, and in $a$ and $r$ means were tested using MANOVA.

The periods of individual rhythms were determined by spectral analysis using both the periodogram method (13, 47) and the linear-nonlinear least squares method (39). A single best period estimate for the animal ($\tau$) was obtained from $T_b$, HP, and HL period estimates, which generally agreed to within one sampling interval. Period estimates were also obtained for activity, feeding, and calculated $C$. We compared $\tau$ between $T_A$ using paired t-tests (SPSS).

Statistically significant ultradian rhythms were also quantified using linear-nonlinear least squares fits, fitting multiple periods. The circadian component was satisfactorily filtered by ignoring significant periods of >18 h. The fractions of total ultradian amplitude, relative to the summed amplitude of ultradian and circadian components, were compared between $T_A$ by evaluating individual differences between 17 and 27°C using MANOVA.

Cumulative sums over the course of average rhythms and average rhythm totals were calculated for HP, HL, and feeding. Rates of HP and HL (in W/kg) were converted to kJ/kg by assuming data at 10-min sample intervals approximated an average over the subsequent interval. Total activity was also calculated from average waveforms at each $T_A$. Cumulative activity was calculated as proportion of total average rhythm activity for each animal at each $T_A$. Differences in total HP and HL, and feeding between $T_A$s were tested by MANOVA.

RESULTS

Rhythms in thermoneutrality. Figure 1 shows three days of data from a monkey in thermoneutrality (27°C).
Means during a monkey. However, Tb duringpared with thermoneutrality, as it was in two otherslightly elevated in this monkey (37.5–38.5°C) com-
MANOVA (Pillais' trace during mild cold exposure (TAanimal is awake at these times.
Brief episodes of elevated HP, HL, and C are also seenperiods, similar to the pattern in thermoneutrality.
Increases in HP were seen, showing greater elevation than duringas during
Feeding and activity counts indicate periodic arousal
periods, and rhythm minimaaveraged from six mon-
periods, and rhythm minima and maxima are summarized in Fig. 4A, as well asaverage rhythm amplitudes (Fig. 4B) and (Fig. 4C).
Rhythm means showed a significant effect of TA inMANOVA (Pillais' trace = 0.99, F5,1 = 346.1, P < 0.05).
Means during α also showed a significant effect of TA(Pillais' trace = 0.99, F5,1 = 1,806.4, P < 0.05), as didmeans during ρ (Pillais' trace = 0.99, F3,1 = 488.4, P <0.05). Rhythm minima were significantly affected by TA(Pillais' trace = 0.99, F4,2 = 45.1, P < 0.05). Feedingminima were omitted from the comparison, because allwere zero. Rhythm maxima did not show a significanteffect of TA overall (Pillais' trace = 0.99, F5,1 = 122.0, P = 0.069); however, individual F tests showed effects onthermoregulatory effectors, HP, HL, and C. Thefeeding maximum was also significantly elevated in the
cold. Rhythm amplitude estimated by the cosinormethod did not show a significant effect of TA in multivariate tests (Pillais' trace = 0.99, F5,1 = 74.7, P =0.088); however, Tb and C rhythm amplitudeswere significantly altered. Rhythm ranges, calculated fromthe minima and maxima shown in Fig. 4, showed a significant effect of TA (Pillais' trace = 1.0, F4,2 = 383.8, P < 0.01), similarly attributable to changes in Tb and Crhythms.
Fig. 3. Average waveforms (±SE) for 6 monkeys are shown for both thermoneutrality (T_A = 27°C, gray lines) and mild cold (T_A = 17°C, black lines). Phase angle was standardized to each animal's rhythm period (τ) and interpolated before averaging of values for all animals (n = 6). Changes due to T_A include increased amplitude of T_b rhythm, elevated HP and HL, and decreased amplitude of C rhythm. However, rhythm phasing and most rhythm amplitudes are similar.
Fig. 4. A: means ± SE for rhythms, means for active (α) and rest (β) periods, and rhythm minima and maxima. B: mean ± SE rhythm amplitudes. C: mean ± SE τ. Means at 27°C are shown by light hatching and means at 17°C by dark hatching. Statistically significant differences between TA are indicated (*P < 0.05; **P < 0.01).
Mean ± SE Tb at 27°C was 37.88 ± 0.09°C and at 17°C was 38.08 ± 0.20°C. Individual differences between TA were not significant in a univariate F test. On average, Tb during α was 38.69 ± 0.22°C at 17°C and 38.36 ± 0.07°C at 27°C, but differences were not statistically significant. Tb during ρ was 37.24 ± 0.21°C in mild cold and 37.29 ± 0.10°C in thermomeutrality, and the difference also did not reach statistical significance. Maximum Tb at 27°C was 38.79 ± 0.05°C and 39.05 ± 0.24°C at 17°C. Differences were not significant. Minimum Tb at 17°C (36.81 ± 0.24°C), however, was significantly decreased relative to that at 27°C (37.89 ± 0.09°C, F1,5 = 30.6, P < 0.01). Amplitude of the Tb rhythm was 0.746 ± 0.03°C at 27°C and 0.96 ± 0.05°C at 17°C. Significantly increased rhythm amplitude was seen in the cold (F1,5 = 37.8, P < 0.01). Similarly, the range of the Tb rhythm was significantly greater at 17°C (F1,5 = 134.2, P < 0.001). Individual cycle amplitudes from cosinor analysis showed a slight transient increase in Tb rhythm amplitude over the first five cycles in cold compared with those in thermoneutrality. A linear least squares fit of differences between 17 and 27°C against cycle number demonstrated significantly increasing amplitude (intercept = 0.05, slope = 0.05, Pearson r² = 0.776, P < 0.05). Cycle means showed no significant difference between TA. No other significant differences in either cycle means or amplitudes over time were seen.

Both HP and HL continued to show robust rhythmicity in mild cold and were conspicuously elevated at all phases of their rhythms. Means for both the HP and HL rhythms were significantly elevated at low TA, as expected based on a prior study of Stitt and Hardy (43). In thermoneutrality, mean HP was 4.82 ± 0.20 W/kg, and in mild cold it was 6.16 ± 0.40 W/kg. Differences were statistically significant (F1,5 = 19.8, P < 0.01). Mean HL at 27°C was 4.64 ± 0.21 W/kg and at 17°C was 6.17 ± 0.28 W/kg, and differences were significant (F1,5 = 43.5, P < 0.001).

Increased HP and HL were seen during both α and ρ. Mean α HP was 7.08 ± 0.54 W/kg at 17°C and 5.60 ± 0.30 W/kg at 27°C. Differences were statistically significant (F1,5 = 12.7, P < 0.05). Means during α were also significantly different for HL (F1,5 = 26.7, P < 0.01). At 17°C, mean α HL was 7.00 ± 0.39 W/kg, and at 27°C it was 5.39 ± 0.31 W/kg. Average ρ HP in thermoneutrality was 3.90 ± 0.17 W/kg, and in mild cold it was 5.25 ± 0.26 W/kg (F1,5 = 14.1, P < 0.05). Average ρ HL was 3.79 ± 0.18 W/kg at 27°C and 5.25 ± 0.26 W/kg at 17°C (F1,5 = 32.9, P < 0.01).

Elevated HP and HL were also demonstrated by increases in both minima and maxima. Minimum HP increased significantly from 3.30 ± 0.20 W/kg at 27°C to 4.37 ± 0.29 W/kg at 17°C (F1,5 = 10.8, P < 0.05). Maximum HP was 6.55 ± 0.36 W/kg at 27°C and 8.27 ± 0.70 W/kg at 17°C (F1,5 = 14.1, P < 0.05). Minimum HL at 27°C was 3.06 ± 0.14 W/kg, and at 17°C it was 4.52 ± 0.15 W/kg (F1,5 = 84.2, P < 0.001). Maximum HL was 6.25 ± 0.37 W/kg at 27°C and 8.13 ± 0.49 W/kg at 17°C (F1,5 = 22.4, P < 0.01). Neither amplitudes nor ranges of the HP and HL rhythms differed significantly between TA.

Whole body C was conspicuously reduced at low TA. Animals observed in low TA typically exhibited piloerection and adopted a more hunched posture when resting. The rhythm mean of C in thermoneutrality was 0.44 ± 0.02 W·kg⁻¹·°C⁻¹, and mean C in mild cold was 0.30 ± 0.02 W·kg⁻¹·°C⁻¹. Differences were statistically significant (F1,5 = 70.3, P < 0.001).

Unlike the HP and HL rhythms, the C rhythm amplitude was reduced to be in amplitude at low TA due to disproportionate reduction of α values. Mean C during α was 0.50 ± 0.024 W·kg⁻¹·°C⁻¹ at 27°C and 0.33 ± 0.028 W·kg⁻¹·°C⁻¹ at 17°C (F1,5 = 115.4, P < 0.001). During ρ, mean C was 0.38 ± 0.016 W·kg⁻¹·°C⁻¹ at 27°C and 0.27 ± 0.018 W·kg⁻¹·°C⁻¹ at 17°C (F1,5 = 25.3, P < 0.01).

Minimum and maximum C were significantly reduced in the cold. At 27°C, minimum C was 0.32 ± 0.02 W·kg⁻¹·°C⁻¹ and the maximum was 0.57 ± 0.03 W·kg⁻¹·°C⁻¹. At 17°C, minimum C was reduced to 0.22 ± 0.02 W·kg⁻¹·°C⁻¹ (F1,5 = 19.6, P < 0.01) and the maximum was reduced to 0.39 ± 0.04 W·kg⁻¹·°C⁻¹ (F1,5 = 115.2, P < 0.001). The C rhythm amplitude at 27°C was 0.087 ± 0.018 W·kg⁻¹·°C⁻¹, and at 17°C it was 0.043 ± 0.010 W·kg⁻¹·°C⁻¹ (F1,5 = 9.8, P < 0.05). The rhythm ranges, calculated from minima and maxima, also differed significantly between TA (F1,5 = 8.4, P < 0.05).

For feeding, rhythm means and amplitudes were not significantly different between TA. Mean feeding at 27°C was 2.68 ± 0.27 counts per 10-min interval, and at 17°C it was 3.29 ± 0.44 counts per 10-min interval, not significant different. However, feeding during both α and ρ increased significantly in separate tests of differences between TA. During α at 27°C, mean pellet delivery was 4.1 ± 0.4 pellets per 10-min sampling interval, which increased to 5.6 ± 0.9 pellets per 10-min sampling interval at 17°C (F1,5 = 6.9, P < 0.05). At 27°C, feeding during ρ averaged 0.9 ± 0.1 pellets per 10-min sampling interval, and at 17°C it was 1.3 ± 0.2 pellets per 10-min sampling interval (F1,5 = 16.5, P = 0.01). Increased ingestion and energy intake are thus suggested at low TA, but different wastage of delivered food pellets, although not observed, was not measured. The amplitude of the feeding rhythm appeared to be greater in the cold, 2.61 ± 0.37 counts in 10 min, compared with 1.82 ± 0.18 counts in 10 min in thermoneutrality, but the difference did not reach statistical significance.

The τ estimated from the periodogram method are shown in Fig. 4C. Periods were significantly shorter (P < 0.05 in paired t-tests) at low TA, with similar reductions for Tb (−0.5 ± 0.1 h), HP (−0.4 ± 0.1 h), HL (−0.5 ± 0.1 h), and C (−0.4 ± 0.1 h). Periods did not differ significantly between TA for feeding or activity (not shown). Smaller decreases were seen using estimates from the linear-nonlinear least squares method. Using this method, changes in τ were significant only for Tb (−0.3 ± 0.1 h) and HL (−0.3 ± 0.1 h). Neither method showed significant differences in τ for activity...
or feeding. Differences in estimates are likely attributable to the small number of cycles evaluated.

Onsets of α and ρ and calculated ϕ. Although shorter τ were seen at low TA, evidence for conservation of rhythm internal organization is shown in Fig. 5. Relative phase angles of α onsets, ρ onsets, and ϕ are shown in degrees of each animal’s τ at both TA. The overall organization of rhythms is similar at the two TA. Close coupling of all rhythms is evident, and similar timing relative to the TB rhythm is shown. TA did not significantly affect relative α onsets. Difference in ϕ was significant only for HL (F₁,₅ = 7.96, P < 0.05), showing an advanced peak at low TA. Difference in ρ onsets was significant only for feeding (F₁,₅ = 7.4, P < 0.05).

Ultradian rhythms. Ultradian rhythms were seen for all variables at both TA. Most animals showed significant periods of 12–13 h, most likely due to bimodal α, and significant periods between 2 and 8 h were also seen in some animals. The fraction of total amplitude accounted for by ultradians, although generally higher at 17°C, did not change significantly between TA for any variable. Ultradians accounted for 49% (±3.3) of TB amplitude in thermoneutrality and 43% (±1.6) in cold. For HP, HL, and C, ultradians comprised 54% (±3.0), 53% (±2.7), and 55% (±5.4) of amplitude in thermoneutrality, respectively. In the cold, ultradians increased to 66% (±4.8) of total amplitude for HP, 59% (±1.3) for HL, and 77% (±4.9) for C. Activity and feeding ultradians showed little effect of TA. For feeding, the percentage of total amplitude was 62% at both TA (±4.8 at 27°C, ±4.0 at 17°C). Activity ultradians increased slightly from 58% (±3.3) to 62% (±7.8) at low TA.

Average rhythm totals. Figure 6 shows accumulated totals over the course of average rhythms of HP, HL, and feeding. Also shown is the average time course of fractional activity accumulation, with the total activity in each TA assumed to equal one. Differences showed a significant effect of TA (Pillai’s trace = 0.91, F₃,₅ = 10.0, P < 0.05). Univariate F tests showed significant increases in HP (F₁,₅ = 16.3, P = 0.01) and HL (F₁,₅ = 17.6, P < 0.01) but not in feeding. The time courses of HP and HL accumulation are conspicuously more linear at low TA, reflecting the relative difficulty in regulating HL. Activity accumulation, relative to total rhythm activity, follows strikingly similar time courses in both TA.

DISCUSSION

This study tested the hypothesis that the free-running circadian TB rhythm, in both mild cold and thermoneutrality, is regulated by coordinated changes in both HP and HL rhythms. Altered rhythm properties were also considered as a possible result of altered TB homeostasis. The free-running TB rhythm was shown to result from a phase offset between phase-coupled HP and HL rhythms of approximately equal amplitude in both TA. Although the magnitude of thermal exchange with the environment was substantially increased (HL) and a significant compensatory increase in metabolic rate (HP) occurred, the circadian organization of these thermoregulatory effectors was essentially unaltered at low TA. However, the results suggest that although circadian and homeostatic demands have separate and additive influences on effectors of TB regulation, the CTS acts principally on TB set point.

Homeostatic responses in defense of TB. Transient decreases in TB were observed during the initial acclimation day in at least three monkeys, as reported previously (16). However, after initial cold exposure, defense of TB is evident in this study. Mean TB, measured intra-abdominally, was not significantly changed by cold exposure, although it averaged slightly lower, by 0.2°C, in thermoneutrality. In squirrel monkeys in an LD 12:12 cycle, mean hypothalamic and colonic temperatures showed a tendency to increase with TA (14). In a large monkey, the pig-tailed macaque (Macaca nemestrina), increased TB was also seen with increasing TA (48). Responses of thermoregulatory effectors consistent with TB homeostasis were seen at low TA. As

![Fig. 5. Averages ± SE for relative duration of α, time of α onset, time of ρ end (ρ onset), and time of rhythm acrophases (ϕ; n = 6). Bars indicate α at 27°C (open bars) and at 17°C (shaded bars). Markers show calculated ϕ at 27°C (©) and at 17°C (©). Statistically significant differences between TA are indicated (*P < 0.05).](http://ajpregu.physiology.org/)
expected, whole body HL was increased at all phases of the \( T_b \) rhythm. Compensatory increases in HP were also seen at all phases, and thus total HP was increased with the result that the \( T_b \) rhythm differed little from compared thermoneutrality. Whole body C was reduced at all phases of the \( T_b \) rhythm during cold exposure. Feeding was elevated in the cold, consistent with increased energy demand. Ability of the animals to remain in energy balance was shown by absence of significant body mass changes. Five animals showed a mean \( \pm \) SE body weight increase of 0.6 \( \pm \) 0.8\% over 7 days in thermoneutrality and a decrease of 1.7 \( \pm \) 1.0\% in the cold. Hydration state, which may have changed due to cold-induced diuresis, was not determined.

Circadian rhythm of \( T_b \) is highly conserved in mild cold. Changes in the \( T_b \) rhythm were consistent with prior demonstration of reduced \( T_b \) and greater rhythm amplitude at low \( T_A \) (14). Increased amplitude of the \( T_b \) rhythm at low \( T_A \) was due mainly to reduced \( T_b \). In an earlier study, ranges of hypothalamic and colonic temperature rhythms of squirrel monkeys in an LD cycle were greatest at 20°C, the lowest \( T_A \) tested, and decreased as \( T_A \) approached thermoneutrality (14). In contrast, in one study of humans, the amplitude of the rectal \( T_b \) rhythm was found to decrease with decreasing \( T_A \) between 24 and 20°C but also when \( T_A \) increased between 24 and 32°C (5).

Rhythmicity of metabolism is maintained in mild cold. Rhythms of metabolism have been well documented in many homeotherms and a few poikilotherms (6, 26, 36, 46). In this study, robust rhythms of HP and HL were seen in both thermoneutral and cold \( T_A \). In contrast, a prior study showed variable HP rhythms but highly regular \( T_b \) rhythms in restrained squirrel monkeys in thermoneutrality (18), demonstrating that metabolic rhythms are not necessary for a \( T_b \) rhythm to occur. Humans with metabolic disturbances (hyper- and hypometabolic) also show essentially normal \( T_b \) rhythms (1).

The temporal organization of HP and HL rhythms in this study, as well as the organization of the \( T_b \) rhythm, was largely unchanged in the cold despite altered thermoregulatory demands and elevated metabolism. In a previous study, \( T_b \phi \) was shown to be unaffected by \( T_A \) in squirrel monkeys in an LD cycle (14). In the present study, \( \phi \) of free-running HP, C, or activity or feeding rhythms relative to the \( T_b \) rhythm phase were not significantly different at low \( T_A \). Significantly advanced rhythm \( \phi \) was only seen for HL. Furthermore, onsets of \( \alpha \) or \( \rho \) periods relative to \( T_b \) rhythm phase did not differ significantly between \( T_A \) for HP, HL, C, or activity. Feeding \( \alpha \) onset did not differ significantly, but \( \rho \) onset was advanced in the cold, resulting in a shorter feeding period. In addition, although significantly increased HP and HL were seen at all times at 17°C (cf. Fig. 3), the amplitude of these rhythms did not change significantly, although \( \rho \) HP and HL appeared to be disproportionately increased. These increases generally coincided with intervals in which \( \rho T_b \) was below levels seen in thermoneutrality. A rhythm in C persisted below thermoneutrality, but with decreased am-

![Fig. 6. Cumulative totals for average rhythms (± SE), comparing \( T_A \). Thermoneutrality is shown by gray lines and low \( T_A \) by black lines. HP and HL are shown as total energy expenditure (kJ) based on rates in watts at 10-min intervals. Activity is standardized to fraction of each animal’s total at each \( T_A \).](image-url)
plitude, as seen in prior studies (5, 6, 48). The C rhythm was approximately in phase with the HP and HL rhythms at both TA. Continued rhythmicity of C can be shown to result from rhythmicity of Tb and metabolism at low TA. Reduced amplitude of the C rhythm in the cold relative to the HL rhythm is, however, the result of disproportionate increase in the temperature gradient between Tb and TA compared with the changes in Tb and HP, from which it is calculated. The temperature gradient increased on average 87%, as opposed to increases of 27% in HP and 0.3% in Tb.

Decreased τ in mild cold. Shorter τ may have been a response to TA, as seen by Tokura and Aschoff (45) for the pig-tailed macaque, but this was not systematic for squirrel monkeys (7). In the pig-tailed macaque, shorter τ were seen at 17°C compared with 32°C after step changes in TA. Effects of TA on period have also been reported in other species (3, 20). Because our studies examined neutral and cold TA in separate experiments, other explanations cannot be ruled out, including prior photoperiod, time of year, aging changes, or endogenous annual rhythms. Prior photoperiod was controlled, however, and regressions of τ change against time of year (27°C, February–J uly; 17°C, May, J uly, and September–November) or against aging changes between studies at different TA (1–13 mo) were not significant. It is also unlikely that free-running circannual rhythms would be in the same relative phases at each TA for the five monkeys in this study that showed period changes. Although in this study core Tb was measured intra-abdominally, prior studies showed hypothalamic temperature in squirrel monkeys, although more closely regulated, to decrease in parallel with colonic temperature when TA was reduced (14, 15). The τ changes observed might thus be due to incomplete temperature compensation of the CTS due to slight alterations to the Tb rhythm and hypothalamic temperatures.

Evidence for rhythmicity of Tb set point. Although the mechanism by which the CTS produces the daily Tb rhythm remains unknown, our data clearly indicate that it involves a regulated change in Tb that does not depend on environmental heat load. Aschoff has proposed that the CTS acts primarily on Tb set point in generating the daily Tb rhythm (2). Prior demonstration of an attenuated Tb rhythm increase in rats given antipyretics provides another line of evidence for set point control by the CTS (41). We believe the set point hypothesis is the most parsimonious basis for interpretation of our results.

Relative constancy of Tb is seen during both α and ρ. Both α and ρ Tb are clearly regulated at both TA through autonomic control of both HP and HL. Changes in HP are compensated by changes in HL, and the reverse is also seen. During non-steady-state transitions between α and ρ Tb, we provide evidence for reciprocal augmentation and inhibition of effector, measured here as whole body HP and HL, that is consistent with the classic model of feedback regulation of Tb (10).

Generation of the Tb rhythm by a phase offset between HP and HL rhythms was proposed some years ago by Metz et al. (29). The basis for this phase offset, as well as phase lock with the Tb rhythm can easily be explained through set point change. Excess HP during the daily Tb rise, with inhibited rise of HL, and the reverse, during the daily Tb fall, are consistent with set point change.

Is the Tb rhythm forced or regulated? For either the HP or HL rhythm to drive the Tb rhythm independently from set point change would require failure of compensatory HP and HL changes to maintain regulated Tb. We find no evidence of this in our data, however. At low TA, HL is chronically elevated. However, compensatory offset of HP acts to preserve Tb levels at all times of day, although in severe cold this compensation might not necessarily be successful. In thermoneutrality, we similarly see no evidence for uncompensated effects of HP (or HL) on Tb. Further suggesting a regulated change is the observation that Tb, HP, and HL rhythm timing is largely unchanged in mild cold.

Is HP under circadian regulation? Rhythms of metabolic rate are well known and may be nearly as ubiquitous as Tb rhythms in homeotherms (26). The role of independent rhythms in thermoregulatory effectors remains controversial, however (5, 6, 34, 36, 46). Various physiological rhythms that can alter effector responses have been discussed, including catecholamine (19, 42) and melatonin rhythms (27). An alternative proposal to Tb set point regulation in circadian rhythmicity is regulation of HP or heat content (46). Heat regulation, however, is problematic (11, 25), and there is a general consensus that temperature is sensed and regulated. Reduced TA is expected to reduce peripheral temperatures (48), thus altering heat distribution and content of the body. Nevertheless, the results of this study show that stereotypical rhythms of Tb, HP, and HL occur at low TA as well as in thermoneutrality, despite likely differences in tissue heat distribution and tonically elevated HP and HL. The implication appears to be that although rhythmic variation in HP clearly exists, it does not appear to be easily separable from the regulation of core Tb.

The amplitude of the HP and HL rhythms appears to be conserved between thermoneutrality and mild cold, given otherwise equivalent experimental conditions. However, restraint has been shown to attenuate the metabolic rhythm (18). A circadian rhythm of metabolism may promote energetic economy (8), but at least in squirrel monkeys, it appears to be produced as a part of Tb rhythm generation.

Anti-homeostatic hypothesis: Does circadian variation in thermoregulation "oppose" homeostatic regulation? Phase-dependent thermal preferences and thermoregulatory responses in rodents have been cited as evidence that the circadian Tb rhythm is generated by some unknown mechanism that acts to oppose homeostatic set point regulation (33–35). It has also been suggested that there is a single set point for homeostatic Tb regulation throughout the day and that Tb undergoes forced changes by the CTS, for which counteracting thermoregulatory responses are predicted (35). Although rodents and primates may differ, we do
not find evidence for forced Ta changes in our data, as previously discussed. We believe the antihomeostatic, or non-set point, hypothesis for the Tb rhythm is unsatisfactory for explaining our results on the basis of several additional considerations.

Although Ta differences in our experiment were forced rather than elective, we see no evidence that Ta alters the basic temporal organization of either the Tb rhythm or the rhythmicity of autonomic effectors of Tb regulation at the whole body level. Furthermore, a reexamination of the rodent data (33–35) suggests that although lower Ta are more frequently preferred around the times of maximal Tp, initiation of the rising and falling phases of the Tb rhythm is relatively independent of Ta selection. Thus it is not clear how Ta, as opposed to autonomic changes, relates to the underlying mechanism for circadian Tb rhythm generation.

Phase-dependent responses of Tb to Ta (5, 18, 21, 35, 48), as well as phase-dependent thermoregulatory responses that might influence the response of Tb to Ta, have been described. These include Hp (4, 17, 18, 21, 28, 37), vascular changes (19, 42), skin temperature (18, 48), C, and whole body HL rhythms (4, 5, 37). Changes in effector gain have been thought to reinforce set point regulation (24) and may contribute to generation of the Tb rhythm (38).

Phase-dependent change of metabolic rate with cold exposure has also been described (35), in addition to the tendency for lower ρ Tb. In humans, a wider range of Tb is tolerated during ρ compared with α with triggering homeostatic responses (44). Significantly, our results show that the differential increase in HP during ρ at low Ta coincides with a fall in Tb below the level seen in thermoneutrality. Increased average HL and C are also seen at this time in our data, suggesting that autonomic mechanisms for heat conservation may be compromised, at least in a cold environment, although evidence exists for circadian alteration of related autonomic mechanisms in thermoneutrality, as previously discussed. We again suggest that the most parsimonious explanation is that set point regulation of Tb is active during ρ at low Ta as well as in thermoneutrality, although possibly modified by circadian changes in tolerated Tb range, effector action, or both.

Hierarchical integration of CTS and Tb set point regulation. Evidence from lesion studies (12, 31, 32, 34) shows independent operation of Tb homeostasis and the generation of Tb rhythmicity by the CTS. The results of this study show that CTS organization of thermoregulatory rhythms is relatively independent of homeostatic responses to thermal challenge. The CTS organization of Tb, HP, and HL rhythms appears nearly stereotypical at both Ta and responds little to minor alterations of the Tb rhythm or to homeostatic changes in peripheral effectors. Thus the relationship between the CTS and thermoregulatory systems appears to us to be hierarchical, with set point changes imposed by the CTS on thermoregulatory centers.

Does CTS determine set point rhythm or set point offsets? What is not apparent from this and prior studies showing continued rhythmicity of Tb after preoptic hypothalamic lesions (32, 40) is the nature of the CTS signal producing the Tb rhythm. On the basis of whole body Hp and HL measurements, we can propose, however, that there are both steady-state α and ρ phases and dynamic transitional phases of the Tb rhythm. Indeed, the circadian Tb rhythm might be most simply explained by two set point changes, one occurring at the beginning of α and the other at the beginning of ρ. The rate of Tb increase or decrease at these times, however, is also known to depend on masking factors, as with light and dark masking in squirrel monkeys (37) that produce more rapid transitions compared with LL. The time courses of increased and decreased Tb, HP, and HL in constant conditions do not, however, appear to depend on Tb homeostasis, as shown in the present study. Once elevated (α) or depressed (ρ) Tb is established, it is possible that action of the CTS on Tb set point is no longer required, although other rhythms may continue to act on thermoregulatory effector responses.

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