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BROWN ADIPOSE TISSUE THERMOGENESIS HEATS BRAIN AND BODY AS PART OF THE BRAIN-COORDINATED ULTRADIAN BASIC REST-ACTIVITY CYCLE

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Abstract—Brown adipose tissue (BAT), body and brain temperatures, as well as behavioral activity, arterial pressure and heart rate, increase episodically during the waking (dark) phase of the circadian cycle in rats. Phase-linking of combinations of these ultradian (<24 h) events has previously been noted, but no synthesis of their overall interrelationships has emerged. We hypothesized that they are coordinated by brain central command, and that BAT thermogenesis, itself controlled by the brain, contributes to increases in brain and body temperature. We used chronically implanted instruments to measure combinations of bat, brain and body temperatures, behavioral activity, tail artery blood flow, and arterial pressure and heart rate, in conscious freely moving Sprague–Dawley rats during the 12-h dark active period. Ambient temperature was kept constant for any particular 24-h day, varying between 22 and 27 °C on different days. Increases in BAT temperature (≥0.5 °C) occurred in an irregular episodic manner every 94±43 min (mean±SD). Varying the temperature over a wider range (18–30 °C) on different days did not change the periodicity, and neither body nor brain temperature fell before BAT temperature episodic increases. These increases are thus unlikely to reflect thermoregulatory homeostasis. Episodic BAT thermogenesis still occurred in food-deprived rats. Behavioral activity, arterial pressure (18±5 mmHg every 98±49 min) and heart rate (86±31 beats/min) increased approximately 3 min before each increase in BAT temperature. Increases in BAT temperature (1.1±0.4 °C) were larger than corresponding increases in brain (0.8±0.4 °C) and body (0.6±0.3 °C) temperature and the BAT episodes commenced 2–3 min before body and brain episodes, suggesting that BAT thermogenesis warms body and brain. Hippocampal 5–8 Hz theta rhythm, indicating active engagement with the environment, increased before the behavioral and autonomic events, suggesting coordination by brain central command as part of the 1–2 h ultradian basic rest-activity cycle (BRAC) proposed by Kleitman.

Key words: thermoregulation, temperature, sleep, hippocampal theta rhythm, arterial blood pressure, wavelet mathematics.

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Abbreviations: AP, arterial pressure; BAT, brown adipose tissue; BRAC, basic rest-activity cycle; DWT, discrete wavelet transforms; EEG, electroencephalograph; HR, heart rate; REM, rapid eye movement; SWS, slow wave sleep.

The amplitude of a number of behavioral and autonomic variables suddenly increases in an irregular episodic manner approximately every 1–2 h during the waking phase of the circadian cycle. Since these episodes recur at intervals of less than 24 h they are defined as ultradian events. Although aperiodic in the strictest sense, such events are classified as rhythms, along with circadian (approximately 24 h) and infradian (>24 h) rhythms (Aschoff and Pohl, 1970; Lavie, 1985, 1991; Lloyd and Stupfel, 1991). Variables that exhibit ultradian rhythmicity include behavioral activity, arterial pressure (AP) and heart rate (HR), as well as brown adipose tissue (BAT), body and brain temperatures and metabolic rate (Aschoff and Pohl, 1970; Honma and Hiroshige, 1978; Shimada and Marsh, 1979; Livnat et al., 1984; Alföldi et al., 1990; Stupfel and Pavely, 1990; Lloyd and Stupfel, 1991; Franken et al., 1992a; Closa et al., 1993; Holstein-Rathlou et al., 1995; Lu et al., 2001; Heldmaier et al., 2004; Baker et al., 2005; Lloyd and Rossi, 2008). The amplitude of the episodic increases in autonomic variables is substantial. Brain temperature, for example, may suddenly increase by as much as 1 °C in rats, with the temperature remaining elevated for approximately 20–30 min (Franken et al., 1992a,b; Baker et al., 2005). Ultradian variability may therefore be physiologically important. Previous studies have examined the ultradian rhythmicity of sub-combinations of the behavioral and autonomic variables, but no synthesis of their overall interrelationships has emerged, and the origins and biological significance of the episodic increases are presently obscure.

BAT thermogenesis is one of the autonomic functions that exhibit ultradian rhythmicity (Closa et al., 1993). BAT, regulated by sympathetic nerves as part of facultative thermogenesis, produces heat that contributes substantially to maintenance of body temperature in cold environments in rats (Carey et al., 2003; Cannon and Nedergaard, 2004; Morrison, 2004; Blessing et al., 2006). We hypothesized that ultradian rhythmicity in BAT thermogenesis contributes to similar rhythmicity in body and brain temperatures, independently of the contribution of BAT thermogenesis to thermoregulatory homeostasis. BAT thermogenesis is increased during food intake, so that ultradian rhythmicity in BAT temperature could also be related to eating (Rothwell and Stock, 1979; Himms-Hagen, 1995).

Our study was carried out in conscious freely-moving chronically instrumented Sprague–Dawley rats. In the present paper we report results for the dark active phase of the circadian cycle. By measuring appropriate combinations of variables in different experiments, we examined

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phase-relationships between ultradian rhythms in behavioral activity, AP and HR, as well as BAT, brain and body temperature. We examined the effect on BAT ultradian rhythmicity of varying the ambient environmental temperature (18–30 °C) on different experimental days, with temperature remaining constant for the 24 h of a given experimental day. By measuring brain and body temperatures just before the onset of each episodic increase in BAT temperature, we further investigated whether falls in body/brain temperature could be thermoregulatory triggers for the episodic BAT temperature increases. We measured thermoregulatory blood flow in the tail artery to assess the contribution of heat dissipation to the episodic temperature changes. We also determined whether ultradian rhythmicity in BAT temperature depends on food intake.

Finally we tested the hypothesis that brain central command coordinates the ultradian rhythmicity in all these somatic and autonomic variables. We examined the relationship between ultradian rhythmicity in BAT temperature and the power of theta rhythm (5–8 Hz) in the simultaneously recorded hippocampal electroencephalograph (EEG), an index of vigilance and active engagement with the environment (Sainsbury et al., 1987; Chrobak and Buzsáki, 1996; Vertes et al., 2004). Our studies suggest that BAT thermogenesis, triggered by brain central command, heats the brain and the body during the episodic increases in vigilance and behavioral activity that occur as part of the 1–2 h basic rest-activity cycle (BRAC) proposed by Kleitman (1982).

EXPERIMENTAL PROCEDURES

Animals

All experiments (Sprague–Dawley rats, 200–450 g) were conducted at Flinders University and the University of Indiana (male rats) and at Monash University (female rats) in accordance with the European Community Council Directive of 24 November, 1986 (86/609/EEC), the Animal Welfare Committee of Flinders University, the Monash University School of Biomedical Science Animal Ethics Committee, and the Institutional Animal Care and Use Committee of the Indiana University School of Medicine. The number of animals and their suffering was minimized. Animals were instrumented under general anesthesia (2% isoflurane in O_2, Veterinary Companies of Australia Pty. Ltd., NSW) or with 80 mg/kg ketamine (Parnell Laboratories Aust Pty Ltd, Silverdale, NSW, Australia) and 11.5 mg/kg xylazine (Ilium, Troy Laboratories Pty Ltd, Smithfield, NSW, Australia) i.p., supplemented as required. Analgesia (Carprofen, Pfizer Pty Ltd, West Ryde, NSW, Australia, 0.1 ml s.c.) and antibiotics (Baytril, Bayer Aust Ltd, Pymble, NSW, Australia, 0.1 ml s.c.) were administered, and animals returned to the animal house for at least 1 week before experiments were carried out.

Ambient temperature and recording conditions

During experiments rats were kept in quiet environments, individually caged away from other rats (Flinders University) or individually caged in the presence of other rats (Indiana University and Monash University), with minimal human intrusions. Standard food and water were available *ad libitum*, except for one experiment (see below) in which rats had access to water but not to food. Ambient temperature was maintained at a constant 24 h daily level, with variation between 18 and 30 °C on different days.

Measurement of BAT, body and brain temperature, HR, and hippocampal EEG (Flinders University)

In the first series of experiments (lights on 0700 h, lights off 1900 h, daily ambient temperature constant over 24 h) BAT temperature was measured with a telemetric probe (TA-F40 W/TP, Data Sciences International (DSI) Transoma Medical, St. Paul, MN). The tip of the temperature probe was positioned between and just ventral to interscapular BAT, near Sulzer's vein. The transmitter body of the telemetry probe was placed in the peritoneal cavity or subcutaneously. The telemetered temperature signal was detected with a PhysioTel Receiver (RLA1020, DSI) and converted to an analog voltage signal with a frequency-voltage converter (# 273–0016, DSI). The converted signals were digitized (1 Hz) with an analog/digital converter (MacLab/8 s, ADInstruments, Bella Vista, NSW, Australia) and recorded with Chart (ADInstruments) data capturing software. Body temperature was measured (1 sample per min) via a SubCue temperature probe (SubCue Dataloggers, Calgary, AB, Canada) positioned in the peritoneal cavity. Insulated wires from the two-pin connector on the SubCue probe were connected to a head socket (see below), so that the probe could be reset after downloading the data in each experiment, without removal from the rat. In these rats tail artery blood flow was also measured by a Doppler ultrasonic probe (Iowa Doppler Products, Iowa City, USA) chronically implanted around the base of the tail artery, with wires from the probe passing s.c. to the head piece, and with signal analysis (40 Hz sampling rate) as previously described (Garcia et al., 2001; Rusyniak et al., 2008).

In a second series of rats studied at Flinders University (lights on 1900, lights off 0700, daily ambient temperature constant over 24 h), BAT, body and brain temperatures were simultaneously measured with thermistor probes and recorded with MacLab/8 s (1 Hz sampling rate). In four of these rats we also recorded the electrocardiograph (ECG) via chronically implanted sternal electrodes (TA11 CTA-F40, DSI). Temperature probes were made from thermistors (10 kΩ, beta =3380 at 25 °C, NTH5G10P, Murata, Kyoto, Japan, size 2×1×1 mm after silicone cover) and with an integrated-thermo sensor (LM35, National Semiconductor, Santa Clara, CA, USA, 6×6×4 mm). Probes were sealed with silicone (RTV 3–1744, Dow Corning, Midland, MI, USA), and calibrated with a linear thermometer at multi-points (every 2 °C from 31 to 41 °C) beforehand. In each rat the temperature probes were positioned in BAT (see above), intracranially, via craniotomy, in a dorsal extradural position near the confluence of the sagittal and transverse sinuses, and in the peritoneal cavity. Wires from the temperature probes (and ECG electrodes) were attached to the head socket and the signals were collected with a bridge amplifier (Biomedical Engineering, Flinders University). In five rats from this second series we removed food from the cage for the 12-h lights on period and for the next 12-h lights off period. These rats had continuous access to water.

In a third series of rats (lights on 0700, lights off 1900, daily ambient temperature constant over 24 h) hippocampal EEG and BAT temperature were simultaneously recorded. The distinctive high amplitude hippocampal theta pattern is more amenable to quantification than the corresponding neocortical EEG which shows low voltage high frequency activity during wakefulness (Bland, 1986). Telemetric BAT probes were first implanted as above. Animals were then positioned in a stereotaxic apparatus. Skull burr holes were made and stainless steel screws fixed for anchoring purpose. Reference and ground electrodes were screwed into frontal and occipital bones. Stainless steel guide cannulae (23 G) were positioned bilaterally just dorsal to the dorsal hippocampus, 3.8–4.0 mm caudal to bregma, 1.9–2.1 mm from midline, and 1.8–2.0 mm ventral to the skull. The tip of a Teflon-insulated silver wire was inserted through the guide cannula and positioned 1 mm below the tip of the guide, targeting the CA1 region. The screw and wire electrodes were connected to a

headpiece through a flexible Teflon-insulated stainless steel wire and fixed with dental cement. EEG signals were fed through a voltage-buffer preamplifier (Biomedical Engineering, Flinders University) to a bridge amplifier/filter unit (ML112, ADInstruments) via a swivel cable. The signals were filtered (0.1–100 Hz band pass) with the ML112 bulletin filter and digitalized (200 Hz) with MacLab/8 s. Signals were recorded with Chart software.

Before experimental use, each rat was suddenly transferred to a cold (5–10 °C) environment and only animals showing at least 0.5 °C increase in BAT temperature were used in subsequent experiments. We have previously established that this procedure validates correct position and function of the probe in interscapular BAT (Blessing et al., 2006).

Arterial pressure, heart rate and behavioral activity (Indiana University)

In male rats (reversed light-dark cycle), AP, HR and behavioral activity were measured using a telemetric transmitter (model TA11PA-PXT50; DSI). The flexible catheter of the telemetric transmitter was implanted in the abdominal aorta via the right femoral artery. The transmitter body was placed in the abdominal cavity and sutured to the abdominal wall. Rats were housed in individual cages and allowed to recover from surgery for at least 7 days. Sampling rate was 1 per min. Behavioral activity (arbitrary units) was measured by variations in the amplitude of the telemetered signal as the rat moved around the cage. We did not directly monitor specific behaviors such as eating or drinking.

Measurement of BAT temperature and behavioral activity (Monash University)

The tip of a BAT temperature probe was positioned in BAT as for the first series of animals studied at Flinders University. The transmitter body of the telemetry probe was placed subcutaneously. Activity was measured by variations in the amplitude of the telemetered signal. The telemetered BAT temperature and behavioral activity signals were transmitted via a BCM-100 consolidation matrix to a computerized data acquisition system (Dataquest IV, DSI). Sampling rate was 1 per 2.5 min. Rats (female) were housed in single cages next to the cages of other female rats (lights off 1000 h, lights on 2200 h).

Wavelet-based analysis of physiological records containing ultradian variability

Ultradian episodes in different physiological variables often occur irregularly, sometimes with small amplitude changes that make it difficult to identify physiologically meaningful increases (Aschoff and Pohl, 1970; Lavie, 1985, 1991; Lloyd and Stupfel, 1991; Stupfel et al., 1995; Lloyd and Rossi, 2008). Wavelet-based mathematical procedures (Graps, 1995) analyse different segments of a signal at appropriately different temporal resolutions, closely fitting original signals that exhibit substantial variability in peak amplitude and inter-peak intervals. We used wavelet-based procedures to fit and smooth the original signals (see below). For increases in BAT temperature or AP to be counted as episodes, we specified the amplitude increases to be at least 0.5 °C and 10 mmHg, respectively, and intervals between episodes to be at least 35 min. To assess the effect of the threshold amplitude chosen to define an episodic increase in BAT temperature, we repeated the analysis with minimum threshold increasing in 0.05 °C steps from 0.5 to 1.0 °C.

Original ADInstrument Chart files were exported to IgorPro (WaveMetrics, Lake Oswego, OR, USA, http://www.wavemetrics.com). Discrete wavelet transforms (DWT function, IgorPro) were performed on all raw signals. The DWT function performs the forward wavelet transform, and outputs transform-coefficients. It then zeros all transform-coefficients whose magnitude falls below 10% of the maximum magnitude of the transform, and then performs the inverse transform of coefficients. After the DWT function, signals were further processed to remove DC components and slow drift/fluctuations with a cycle longer than 12 h. Positive peaks in smoothed BAT temperature signals were identified by analyzing the first and second derivatives of the signals and detecting inflection points (FindPeaks function in IgorPro). The onset of each BAT temperature increase was specified as the time of the first minimum value preceding the peak. DWT functions of simultaneously recorded body temperature, brain temperature ($>$0.2 °C), and behavioral activity were then searched for peaks occurring within $\pm$20 min of a given BAT temperature peak.

Peaks in AP were identified using the DWT procedure (as for BAT temperature peaks, see above). Corresponding peaks in HR (derived from the AP trace, minimum rate increase of 20 beats/min) and in behavioral activity were identified within $\pm$20 min of a given AP peak. AP and HR signals have a higher frequency response than temperature signals. We calculated duration of AP episodes as the time between the first minimum in the 25 min period before a peak (onset time) and the first minimum in the 25 min after a peak (end time). The time between a given end time and the next onset time was measured as an indication of the time between episodic increases in AP.

Analysis of hippocampal EEG and its relationship to episodic increases in BAT temperature

Epochs (10.24 s) of EEG were smoothed with a Hamming window, and the magnitude of the FFT was obtained. Power in the 5–8 Hz theta band was expressed as a percentage of total (1–20 Hz) power for each 1 min bin. We selected 60 min segments of BAT temperature records, each commencing 20 min before and concluding 40 min after the onset of each episodic increase in BAT temperature. Corresponding segments of hippocampal EEG were identified. Paired BAT temperature and hippocampal EEG theta power proportion signals were cross correlated (IgorPro correlation function). We also examined the time relationship between hippocampal theta proportion and BAT temperature by calculating the mean value of each 1 min time point in each 60 min segment, and determining the time difference between onset of the increase in BAT temperature and onset of the increase in the proportion of theta power.

Statistical analysis

Group results were analysed with Statview (SAS Institute, Carey, NC, USA), with mean$\pm$SD used for descriptive statistics and mean$\pm$SE for inferential statistics. Student's *t*-test, and factorial and repeated measures analysis of variance (ANOVA) were used, with post-hoc comparison with Fisher's protected *t*-test, with the significance level of the primary ANOVA set at the 0.05 level. Linear regression, Pearson correlation, and cross correlation (Igor Pro) were used to quantify relationships between different signals.

RESULTS

We observed animals throughout both the 12-h light and the 12-h dark segments of the 24-h day, but we report only results obtained during the lights-off dark period, the time when the rat, as a nocturnal animal, is more likely to be awake and active. Ambient temperature was kept constant during any individual 24-h period.

Periodicity of variations in BAT temperature

As exemplified in the original recordings from individual rats and in the curves fitted with wavelet analysis (Fig. 1),

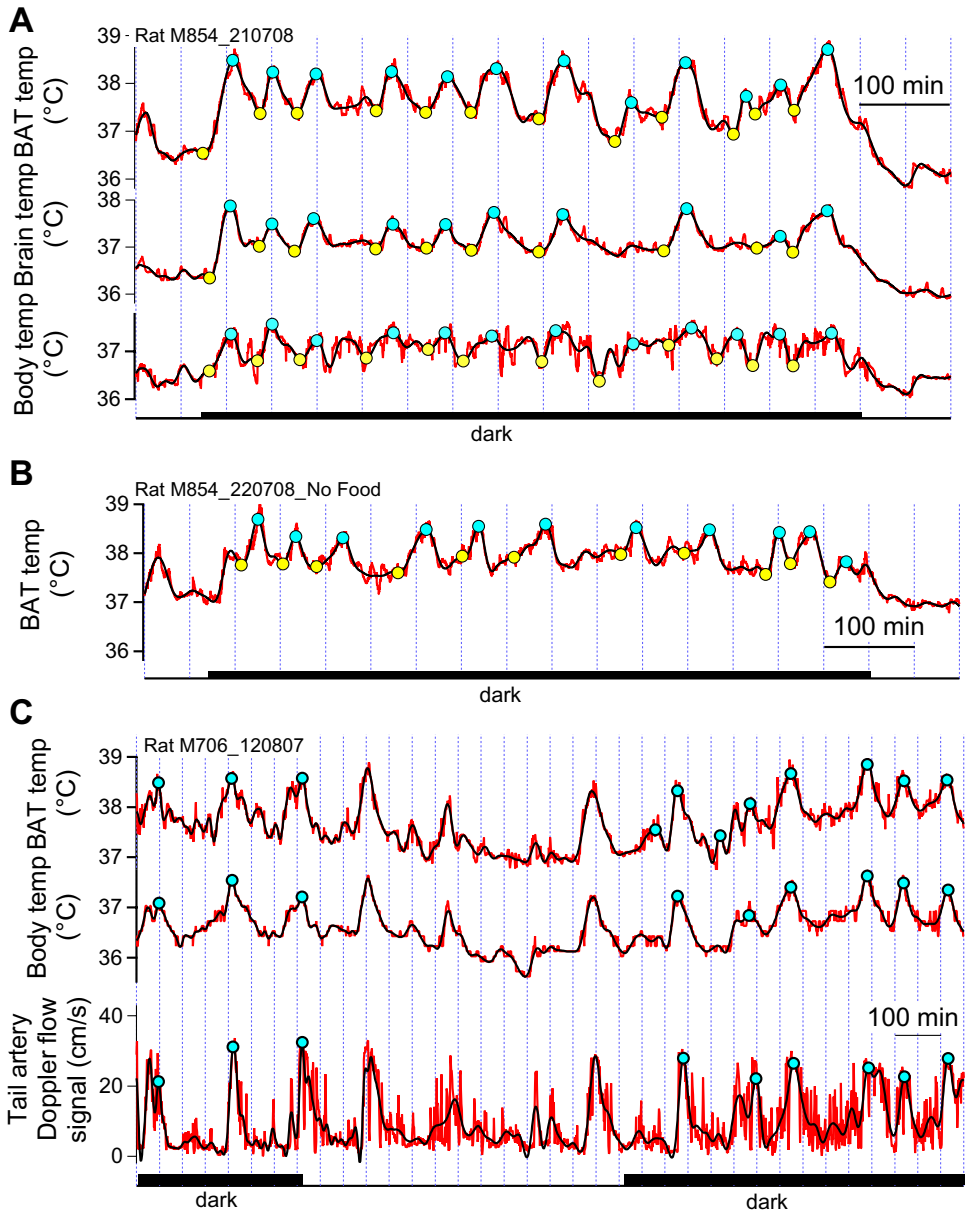

Fig. 1. (A) BAT temperature (top red trace) and brain temperature (middle red trace) and body temperature (bottom red trace) records in one rat. Ambient temperature 23 °C. (B) BAT temperature (red trace) record in one rat without access to food for 12 h before the dark period and for 12 h during the dark period. Ambient temperature 24 °C. (C) BAT temperature (top red trace), body temperature (middle red trace) and tail artery Doppler blood flow signal (bottom red trace) records in one rat. Ambient temperature 26 °C. All original records are 1 min averages. Thicker black lines were fitted by the DWT wavelet function. Blue-filled and yellow-filled black circles indicate peak and onset times. Black bars represent dark lights-off time. The beginning and end portions of the records in (A) and (B), and the middle portion in (C) are from the lights-on time, and both circadian and ultradian periodicities are evident. Only dark (lights-off portions) of the records are analysed in our paper.

BAT temperature increased episodically in association with increases in body and brain temperature. With the threshold amplitude set at 0.5 °C and the ambient temperature 22–27 °C (constant over 24 h), episodes of BAT thermogenesis occurred every 94±43 min (range 35–272 min), amplitude 1.1±0.4 °C and time from onset to peak 34±17 min (mean±SD of 532 episodes in 40 rats). Varying the ambient temperature between 18 and 30 °C on different days had no effect on BAT temperature peak interval (regression $F_{1,199}=1.29$, $P>0.05$), a significant but small effect on BAT temperature peak amplitude (regression $F_{1,221}=47.12$, $P<0.0001$, $R^2=0.18$), and no effect on baseline body temperature measured at the onset of episodic increases (regression $F_{1,187}=1.15$, $P>0.05$).

Frequency distributions of peak intervals and amplitudes for the 0.5 °C threshold definition are shown in Fig. 2A, B and the distribution of peak intervals for a 1.0 °C threshold definition are shown in Fig. 2C. As the threshold amplitude for definition of an episode is increased in 0.05 °C steps from 0.50 to 1.0 °C, the peak interval gradually increases (Fig. 2D).

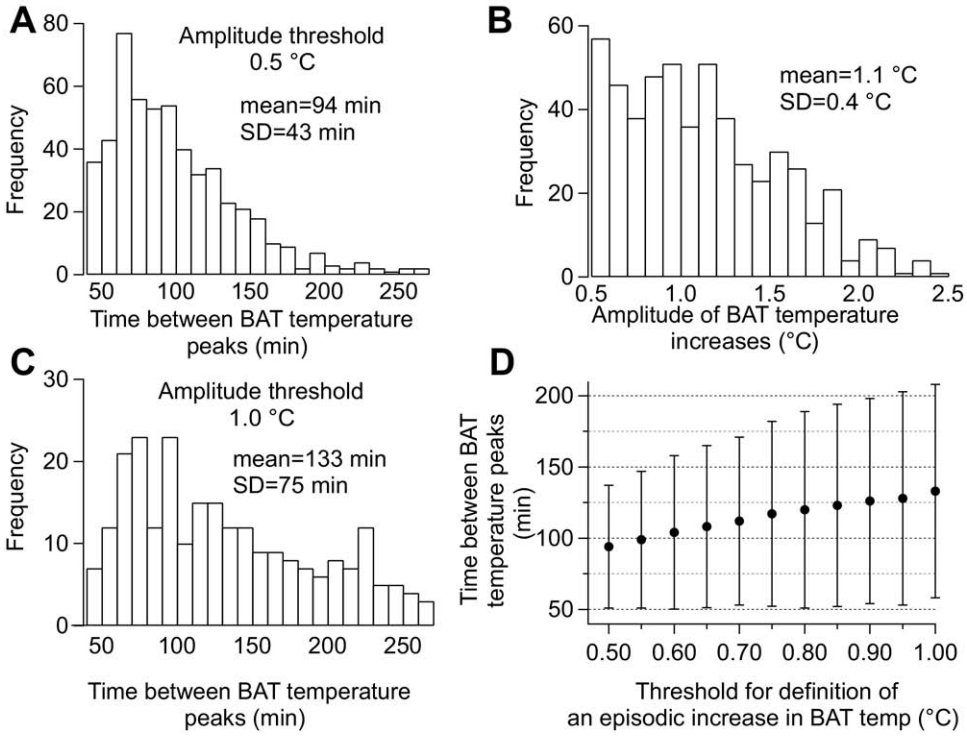

Fig. 2. (A), (B) Frequency distributions of time between peak of episodic increases in BAT temperature at threshold amplitude 0.5 °C, and of amplitudes of the peaks at this threshold. (C) Frequency distributions of peak intervals at threshold amplitude 1.0 °C. (D) BAT peak intervals (mean±SD) with threshold amplitudes increasing in 0.05 °C steps from 0.50 to 1.0 °C. Linear regression between threshold amplitude and peak intervals for all dark period peaks in all rats at ambient temperature 22–27 °C was significant ($F_{1,4295}=161.6$, $P<0.0001$), but the relationship was not strong ($R^2=0.04$).

Relationship of brain and body temperature increases to BAT temperature increases

In rats in which BAT, brain and body temperatures were measured simultaneously, all three temperature probes were similarly constructed, and the sampling rate was 1 Hz. Thus the temperature signals could be closely compared in these animals. We calculated the mean BAT, brain and body temperatures for each rat for the 10 min periods before and after the onset time of each episodic increase in BAT temperature. Ambient temperature was 22–27 °C, constant on any particular day. Results are shown in Fig. 3A where it is clear that brain and body temperatures were at a stable baseline level preceding the onset of episodic increases in BAT temperature. There were no falls in brain or body temperature just before the increases in BAT temperature. Indeed, analysis of corresponding episodic onset times showed that BAT temperature started to increase 2.3±0.62 min before brain temperature (mean±SE, paired $t_{191}=3.71$, $P<0.001$), and 3.0±0.9 min before body temperature (mean±SE, paired $t_{187}=3.35$, $P<0.001$). The increases in BAT temperature were greater than the corresponding increases in brain and body temperatures (1.1±0.4 °C, 0.8±0.4 and 0.6±0.3 respectively mean±SE), with significant and substantial linear regression between corresponding increases in BAT and brain temperatures, and BAT and body temperatures (Fig. 3B, C). The mean correlation between dark period BAT and brain temperature signals was 0.9±0.1, and between BAT and body temperature signals the mean correlation was 0.7±0.2 (mean±SD, 18 dark periods in 13 rats).

Tail artery blood flow

During the onset and initial stages of the bursts in BAT thermogenesis the tail artery vascular bed was constricted (Fig. 1C, bottom trace). Tail artery vasodilatation occurred abruptly 15±1 min (mean±SE) after onset of each BAT temperature increase, with resumption of tail vasoconstriction 7±1 min (mean±SE) after the peak of each BAT temperature increase.

BAT temperature, behavioral activity, AP and HR

The DSI probe (see Methods) measures general behavioral activity as a function of variations in the strength of the telemetered signal as the animal moves towards and away from the signal detector. Most episodes of BAT thermogenesis occurred together with an increase in behavioral activity measured in this manner (Fig. 4A). Comparison of onset times of corresponding episodic increases in activity and BAT temperature in the DWT traces showed that activity commenced 3±1 min before BAT temperature increases (mean±SE, paired $t_{212}=3.75$, $P<0.001$). The mean maximum correlation between the two signals was 0.8±0.02 (mean±SE, $n=25$ records in 14 rats).

As shown in Fig. 4B, both AP and HR exhibited periodic increases in amplitude (18±5 mmHg and 86±31 beats/min, mean±SD, $n=161$ peaks in 10 rats), with a

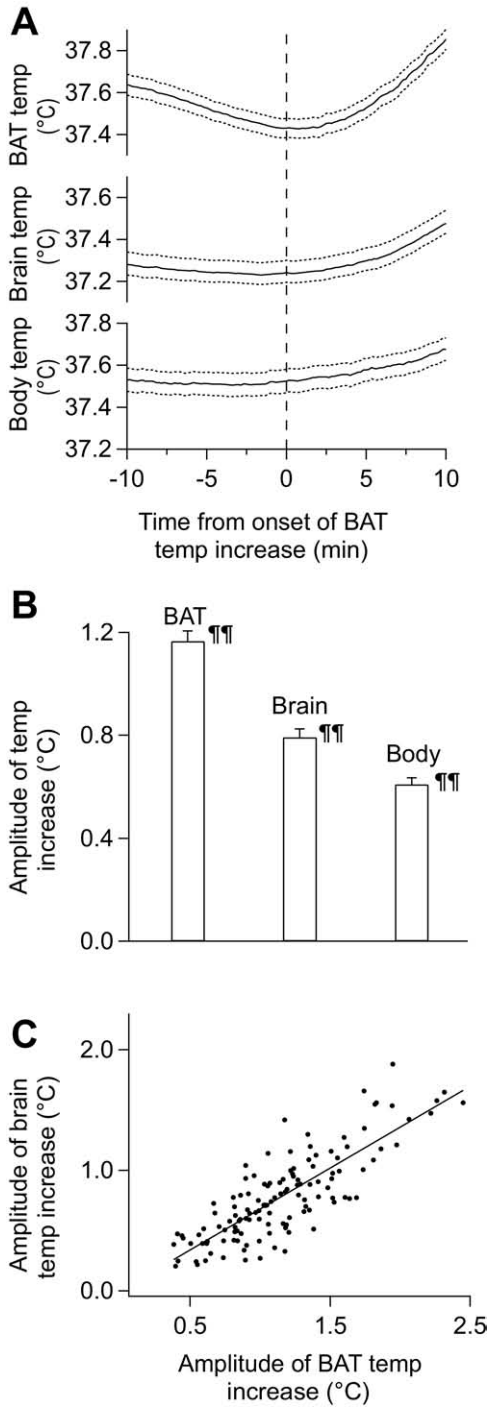

Fig. 3. (A) Body and brain temperatures for the 10 min periods before and after the onset time of ultradian episodes in BAT temperature (mean±SE of 121 episodes). (B) Amplitudes (mean±SE) of BAT, brain and body temperature increases during the dark period; ambient temperature 22–27 °C. ¶¶ each mean is significantly different from the other two (repeated measures $F_{2,182}=208.9$, $P<0.0001$). (C) Linear regression between amplitudes of corresponding increases in BAT and brain temperature (regression $F_{1,190}=224.8$, $P<0.0001$, $R^2=0.54$).

mean AP peak interval of 98±49 min (mean±SD, $n=156$ peaks in 10 rats). The distribution of AP peak interval times and amplitudes is shown in Fig. 5A, B. Cross correlation between AP and HR traces for the dark period showed no significant time delays between the signals, with a correlation of 0.9±0.04 (mean±SD, $n=22$ traces in 10 rats). Regression between the amplitudes of corresponding increases in AP and HR was also significant (regression $F_{1,157}=126.5$, $P<0.0001$, $R^2=0.45$).

Peaks in AP and HR occurred in close relationship to peaks in behavioral activity (Fig. 4B). Cross correlation showed no significant time delays in AP and activity signals. The correlation between the signals was 0.6±0.13 (mean±SD, $n=22$ signal traces in 10 rats). In separate rats ($n=4$) in which HR and body temperature were measured simultaneously (1 Hz), onset of increases in HR occurred approximately 7±2 min before the onset of the corresponding increase in body temperature (mean±SE, paired $t_{29}=3.14$, $P<0.001$ versus zero delay), and this indicates that the increases in HR (and by implication the increases in AP) occur approximately 4 min before each corresponding increase in BAT temperature.

If the AP signal was oscillatory in the conventional sense, the time between end of one episode and onset of the next episode (off-time) should be close to zero. Zero values did occur (see Fig. 4B), but the mean±SD of the off-times was 34±42 min (150 episodic increases in AP), documenting that intervals between episodes of increased AP occupy approximately one-third of the total dark period time, and that sudden increases in AP may occur from a reasonably stable baseline.

Effect of lack of access to food on episodic increases in BAT temperature

Episodic increases in BAT temperature also occurred in rats without food for a 24-h period (Fig. 1B). The interpeak interval was reduced (70±4 min without food versus 86±3 min with food, $F_{1,244}=7.01$, $P<0.01$). The amplitude of episodic increases in BAT temperature was also slightly reduced (0.93±0.1 °C without food versus 1.10±0.03 °C with food, $F_{1,252}=5.90$, $P<0.02$).

BAT temperature and theta (5–8 Hz) power in the hippocampal EEG

BAT temperature and hippocampal EEG signals were simultaneously measured in a series of rats. The proportion of theta rhythm power (5–8 Hz) in the hippocampal EEG was expressed as 1 min averages as shown in Fig. 6A. Episodic increases in BAT temperature, and their onset times, were identified as described in Methods. We selected 60 min segments of the BAT record (20 min before onset and 40 min after onset of a BAT temperature increase), together with the EEG theta power proportions for the same time periods. An example of a cross correlation of 1 min BAT temperature and EEG theta proportion signals is shown in Fig. 6B. In this example the highest correlation (0.92) occurs when the BAT signal is advanced in time by 5 min. Group results showed that an increase in EEG theta power preceded the episodic increases in BAT temperature by 6.3±0.6 min (mean±SE, $t_{44}=11.1$, $P<$

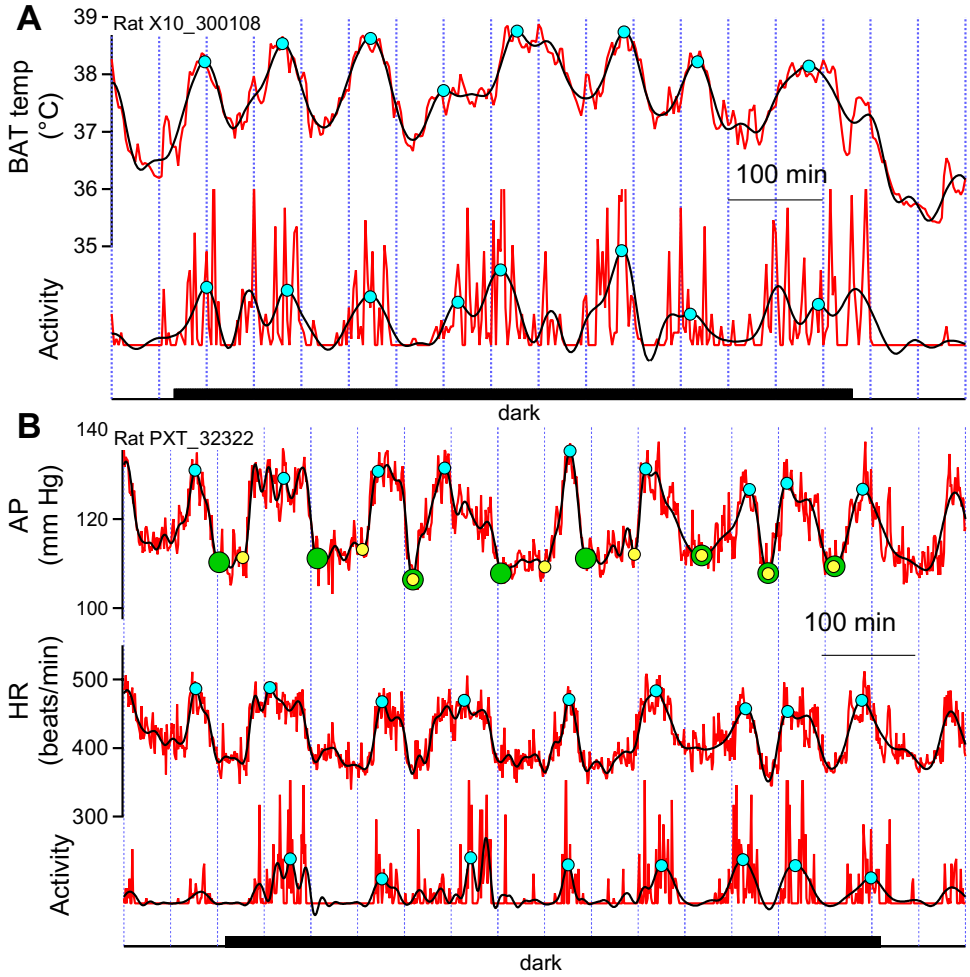

Fig. 4. (A) BAT temperature (top red trace) and behavioral activity (bottom red trace) recorded every 2.5 min in an individual rat. Ambient temperature 24 °C. (B) Mean AP (top red trace), HR (middle red trace) and behavioral activity (bottom red trace) recorded every 1 min in an individual rat. Ambient temperature 24 °C. Blue-filled and yellow-filled circles indicate peak and onset times, respectively. The yellow-filled and green-filled black circles on the AP (top) trace in B indicate peak onset times and peak end times respectively. Coincident end and onset times are shown as yellow circles overlapping the green circles. The black lines were fitted by the DWT function.

0.0001 versus zero time difference). After adjustment for the time difference the correlation between the BAT and the EEG theta power signals was 0.6±0.2 (mean±SD, n=45 segments in eight rats). As can be seen in Fig. 6C, the onset of the increase in mean EEG theta power signal preceded the onset of the increase in mean BAT temperature by about 4–5 min, in reasonably close agreement with the delay determined by cross-correlation of individual signals.

The position of hippocampal EEG recording electrodes was confirmed by post-mortem histological analysis in each rat, with appropriate localization as shown in Fig. 7.

DISCUSSION

Our study confirms and substantially extends the previous report of an ultradian rhythm in BAT temperature (Closa et al., 1993). Episodic increases in BAT temperature were of greater amplitude than the corresponding increases in body temperature, even though blood flowing to BAT increases during the episodic increases (Closa et al., 1993). In anesthetized rats, changes in BAT temperature are associated with corresponding changes in BAT sympathetic nerve discharge (Morrison, 2004; Nakamura and Morrison, 2008; Rusyniak et al., 2008). We thus consider it reasonable to assume that the episodic increases in BAT temperature observed in our study reflect increases in BAT metabolic thermogenesis. BAT can increase its metabolic rate many times, so that, although the tissue constitutes only about 1% of body weight, increases in BAT metabolism can substantially increase whole body metabolic rate (Cannon and Nedergaard, 2004). Thus increases in BAT metabolism could contribute substantially to the well-documented ultradian rhythmicity in whole body metabolic rate in rats during the dark active phase of the circadian cycle (Stupfel and Paverly, 1990; Stupfel et al., 1995). Characteristics of the ultradian rhythm in BAT thermogenesis are described later in the Discussion section.

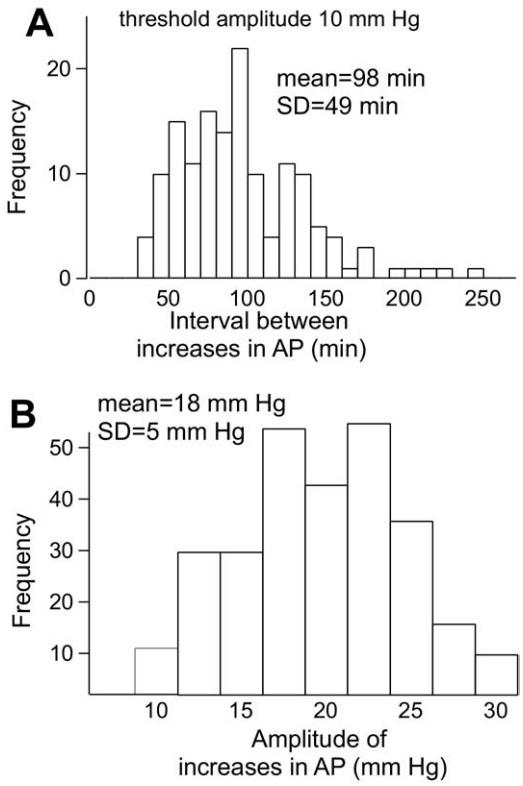

Fig. 5. Frequency distributions of peak intervals (A) and amplitudes (B) of episodic increases in AP (threshold amplitude 10 mm Hg).

The brain initiates and coordinates episodic increases in behavioral activity, BAT thermogenesis, AP and HR

We demonstrate, for the first time, that episodic increases in BAT, brain and body temperature are phase-linked with episodic increases in behavioral activity, AP and HR. The episodic increases in BAT temperature were preceded by increases in hippocampal theta (5–8 Hz) power. Hippocampal theta rhythm is a recognized marker for vigilance and active engagement with the environment (Bland, 1986; Sainsbury et al., 1987; Chrobak and Buzsáki, 1996; Vertes et al., 2004). After correction for the time difference, hippocampal EEG and BAT temperature signals were highly positively correlated during individual episodic increases in BAT temperature. Thus our results confirm our hypothesis that brain central command initiates and coordinates all the phase-linked episodic somatic and autonomic processes as part of the animal's episodic active engagement with the environment. Other investigators have demonstrated episodic occurrence and phase-linking of different combinations of the processes we measured, but we believe that our study provides the first experimental evidence that they are all phase-linked by brain central command.

Ultradian rhythms in body and brain temperature are more obvious after lesions of the suprachiasmatic nucleus that abolish circadian rhythms (Baker et al., 2005; Fuller et al., 2008), so that ultradian rhythms must be determined by other brain regions. At present these regions and the presumed gene-regulated clock mechanisms that generate the phase-linked ultradian rhythmicity are unknown. Hippocampal theta rhythm and the sleep/wake cycle reflect complex forebrain events that are influenced by brainstem, hypothalamic and basal forebrain neural circuits (Kapás et al., 1996; Chrobak and Buzsáki, 1996; Vertes et al., 2004; Saper et al., 2005). Perhaps these circuits also control phase-linked ultradian rhythmicity in autonomic and behavioral variables, including BAT thermogenesis. Descending neural pathways to the relevant spinal sympathetic outflows controlling BAT thermogenesis relay in the hypothalamus and in the raphe/parapyramidal region of the medulla oblongata (Bamshad et al., 1999; Oldfield et al., 2002; Cano et al., 2003; Morrison, 2004).

The precise sequence of brain-coordinated somatic and autonomic events needs further investigation. The exact onset of episodic behavioral activity is often difficult to specify and in our study the sampling rate for activity was only 1 per 2.5 min. The delay between increase in theta power and onset of the increase in BAT temperature may partially reflect the slow frequency response of the temperature signal. It is unlikely that the delay indicates that episodic BAT thermogenesis is principally controlled by adrenal catecholamine secretion because there are 80–90 min ultradian peaks in plasma noradrenaline in rats, without corresponding peaks in plasma adrenaline (Tapp et al., 1981).

Episodic increases in BAT temperature are unlikely to reflect a homeostatic thermoregulatory process

In conscious rats, sudden transfer to a cold environment triggers vigorous BAT thermogenesis within minutes (Blessing et al., 2006). Thus, as part of thermoregulatory homeostasis in conscious rats, ultradian increases in BAT thermogenesis could be triggered by falls in skin/body/brain temperature. However in our study there was no fall in either body or brain temperature just before each episodic increase in BAT temperature. Moreover when we varied the 24 h ambient temperature between 18 and 30 °C (constant during a particular day) there was no change in the periodicity of BAT thermogenesis and no change in the body temperature at the onset of each episode. Although the ambient environmental temperature was varied on different days, it was kept constant for the 24 h period on any particular day, so that skin temperature receptors should not have been activated by changes in ambient temperature. Thus episodes of BAT thermogenesis are unlikely to be triggered by feedback from thermoreceptors as part of thermoregulatory homeostasis. Similarly, as also noted by Shimada and Marsh (1979), episodic coupled increases in AP and HR (with a positive 0.9 correlation in our study) are unlikely to reflect activation of peripheral baroreceptors as part of arterial pressure homeostasis. Baroreceptor activation by increases in AP should cause a bradycardia.

Preoptic neurons may have altered thermosensitivity during wakefulness as compared to sleep (Parmeggiani et al., 1986; Baker et al., 2005; Szymusiak and McGinty, 2008), so that BAT thermogenesis could be activated at the beginning of an active wakeful period at the ambient temperature that was previously ineffective in activating

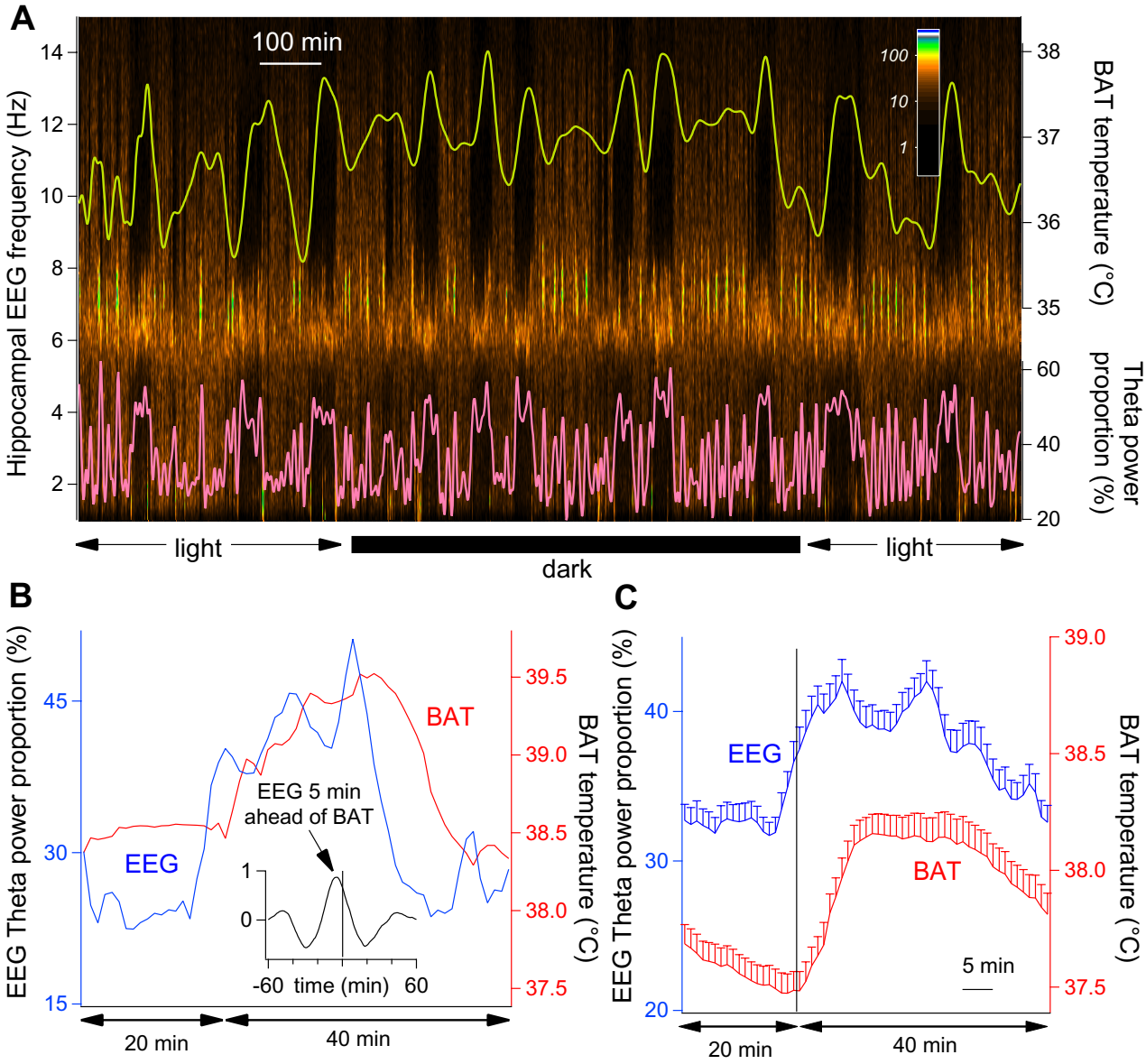

Fig. 6. (A) Left axis and Z-axis: hippocampal EEG power spectra (Fourier transformation of raw EEG signal, magnitude-squared, 10.24 s epochs, 1 min bins), color coded (log scale) as in inset at top right. Right axis, upper trace: BAT temperature (means of 1 min bins). Right axis, lower trace: Percentage of hippocampal EEG power in 5–8 Hz theta band in relation to total power (1 min bins). (B) EEG theta power proportion (percent of total power) and BAT temperature (left blue and right red axes respectively) for the period 20 min before and 40 min after the onset of an ultradian increase in BAT temperature (1 min bins). The inset shows the result of cross correlation of the EEG and BAT signals (see text). (C) Group data (mean±SE) for percentage of 5–8 Hz theta EEG power (blue left vertical axis) from the same dark period segments as shown for the BAT temperature (red right vertical axis) obtained from 60 min dark period segments, each commencing 20 min before an ultradian episode of BAT thermogenesis, marked by the vertical line (1 min bins). Data from 45 segments in eight rats. The increase in EEG theta power commences approximately 5 min before the BAT temperature signal starts to increase.

BAT thermogenesis. We consider that the concept of a changing "set-point," implicit in this manner of explanation, is not a helpful way of understanding the episodic events that we observed, especially given their large amplitudes. The origin of all the ultradian autonomic events is illuminated by our proposal that they are initiated and coordinated by brain central command. This is not to deny the physiological importance of inputs to the brain from peripheral receptors. The increase in tail blood flow, commencing approximately 15 min after the onset of each episodic increase in BAT temperature, as body and brain temperatures increase, may well reflect heat dissipation via thermoregulatory homeostatic mechanisms. Peripheral inputs are integrated into the overall physiological response.

BAT thermogenesis contributes to episodic increases in brain and body temperatures

Episodic increases in brain temperature during the active period of the circadian cycle have previously been reported for rats and other species (Hayward and Ball, 1966; Baker and Hayward, 1967; Hayward and Baker, 1968, 1969;

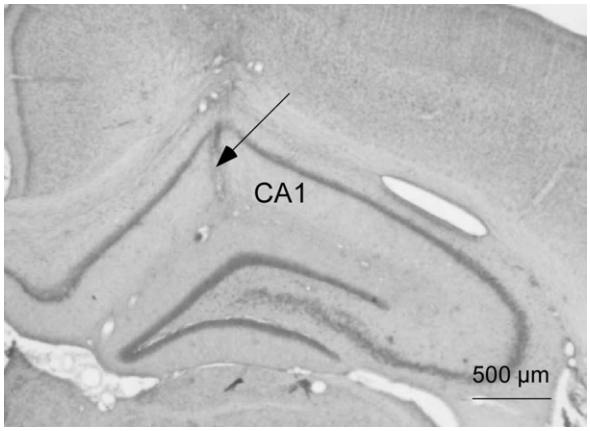

Fig. 7. Neutral red stain of a coronal brain section showing a typical location of the EEG recording electrode in the hippocampal CA1 area (arrow).

Alfödi et al., 1990; Franken et al., 1992a,b; Baker et al., 2005). An increase in the temperature of the arterial blood supplying the brain is a significant contributor to the increases in brain temperature (Hayward and Baker, 1968, 1969), suggesting a peripheral source of heat production. Skeletal muscle contraction associated with peaks in behavioral activity produces heat, but the temperature increases sometimes occur in the absence of activity, and the heat produced in muscle during activity is insufficient to account for the temperature increases (Honma and Hiroshige, 1978; Eastman and Rechtschaffen, 1983; Refinetti, 1994; Girardier et al., 1995; Decoursey et al., 1998).

The normal physiological function of BAT thermogenesis is to heat the rest of the body. In our study the onset of the BAT temperature increases, 2–3 min before the corresponding increases in body and brain temperature, together with the larger amplitude of the BAT increases and the strong regression relationship between BAT/body and BAT/brain temperature increases, strongly suggests that BAT thermogenesis contributes to the temperature increases of the other organs. In agreement with this conclusion, subcutaneous administration of SR59230A, a beta-3 adrenoreceptor antagonist, significantly reduces BAT, body and brain temperatures (Y. Ootsuka, R. de Menezes and W. Blessing, unpublished observations). The brain's own metabolism also makes a substantial contribution to its increase in temperature (Kiyatkin, 2005).

Periodicity of increases in BAT temperature and AP; discrete events rather than true oscillations

Peaks in BAT temperature and AP occurred quite irregularly and with variable amplitudes. Standard deviations of interpeak intervals and amplitudes were approximately 50% of the mean values, with substantial intra-animal variation. The AP records show numerous instances of rapid increase from a relatively stable baseline, continuation at a plateau level, and then rapid return to the baseline (see for example, the square wave appearance in the first and second dark period increases in AP in Fig. 4B). BAT temperature also sometimes exhibited sudden increases from relatively stable baselines. The episodic variations in both temperature and cardiovascular function thus have the characteristics of discrete events rather than true sinusoidal oscillations. The large variability of peak amplitudes and intervals is characteristic of ultradian events in many other physiological variables. Although ultradian cycles are aperiodic in the strictest sense, episodes rather than true oscillations, they have been classified as "rhythms," along with infradian and circadian rhythms (Aschoff and Pohl, 1970; Lavie, 1985, 1991; Lloyd and Stupfel, 1991). The episodes occur at variable intervals, consistent with our conclusion that they are physiological responses integrated with the animal's periodic behavioral interaction with the environment.

We consider that 0.5 °C increases in BAT temperature and 10 mmHg increases in AP are reasonable threshold criteria for defining episodic increases. Changes of these magnitudes are significant in many physiological situations. The highly significant regressions between corresponding increases in BAT and brain temperature, and between AP and HR, indicating that even small "blips" in the BAT and AP temperature record correspond with similar "blips" in the other records, attests to physiological processes rather than measurement error or random variations. With these criteria, selected before we performed our analyses, BAT interpeak interval was 94±43 min and AP interpeak interval was 98±49 min. We recognize that selection of different threshold criteria results in different mean interpeak intervals (see Fig. 2D), but in view of the large standard deviations the issue of the "exact periodicity" is not a crucial one.

Increases in AP and HR may facilitate distribution of heat from BAT to brain and body

Ultradian increases in AP, HR and cardiac output, of substantial amplitude and with peak interval approximately 100 min, were first described in conscious dogs (Shimada and Marsh, 1979; Livnat et al., 1984). Episodic increases in AP quite similar to those in our report have also been documented in rats (Holstein-Rathlou et al., 1995). Scant attention has been paid to the physiological significance of these cardiovascular events in spite of their magnitude. In rats, blood flow to BAT is substantially increased during periods of increasing BAT temperature (Closa et al., 1993). Thus the cardiovascular changes may facilitate rapid transfer of heat from BAT to the brain and to other bodily organs metabolically active during periods of behavioral activity.

Episodic increases in BAT temperature are not dependent on food intake

Rats with continuous access to food nevertheless eat episodically, about 9–12 times per day, mostly during the dark phase and in association with increases in body temperature (Himms-Hagen, 1995). The temperature increases (diet-induced thermogenesis) partially reflect BAT thermogenesis (Rothwell and Stock, 1979). Although we did not specifically measure food intake, this activity is presumably an important contributor to the activity increases recorded

in our study. Episodic increases in BAT temperature are not dependent on food intake since, as we demonstrated, the episodes still occur in rats without access to food. The 9–12 per day frequency of the episodes of eating (mostly during the waking period) is illuminated by the idea that eating is one of the important behaviors that occur during the active phase of the basic rest activity cycle (BRAC) (see below). When food is not available the more urgent search for food could shorten the periodicity of the BRAC, with reduced amplitude of BAT thermogenic episodes as demonstrated in our study. The timing and causal relationships between food intake and BAT thermogenesis are not fully documented and further studies are required. BAT "diet-induced thermogenesis" might be part of the general integrative physiology of the active phase of the BRAC rather than being specifically related to food intake.

BAT thermogenesis heats the brain as part of the ultradian basic rest activity cycle (BRAC)

Prompted by the discovery of rapid eye movement (REM) sleep in humans, Kleitman proposed that the BRAC, a 1–2 h ultradian periodicity in alertness and behavioral activity, is a fundamental part of mammalian life (see Kleitman, 1982 and a review in Lavie, 1991). Episodic REM sleep was interpreted as the manifestation of BRACs during the sleep phase of the circadian cycle, with behavior prevented by sleep paralysis. Alternation between REM and non-REM brain states during the inactive part of the circadian cycle is now an accepted part of physiological knowledge. There are physiological similarities between alert brain states associated with behavioral activity and the paradoxical "brain awake" state of REM. Hippocampal theta activity and brain temperature also increase during REM sleep in many species (Kawamura and Sawyer, 1965; Baker and Hayward, 1967; Hayward and Baker, 1968, 1969; Alfödi et al., 1990; Franken et al., 1992a,b; Chrobak and Buzsáki, 1996; Vertes et al., 2004; Baker et al., 2005).

Nevertheless the occurrence of the BRAC during the wakeful half of the circadian cycle has been more difficult to document, and this form of ultradian rhythmicity is usually omitted from textbooks of physiology. Our study in rats describes the dark half of the circadian cycle, the period when the animals are more likely to be awake. The episodic increases in BAT temperature, together with increases in body and brain temperature, and with increases in AP and HR, occurred together with behavioral activity, thus during alert wakefulness. A reasonable interpretation of these phase-linked events is that they represent integrated components of the active phase of the BRAC during the waking portion of the circadian cycle. Feedback from the external and internal environments modulates BRAC-related behavioral and autonomic events, but their principal organization derives from brain central command.

The brain warming associated with hippocampal theta activity and BAT thermogenesis could facilitate the complex brain functioning necessary for interacting with the environment during the active phase of the BRAC, an idea previously advanced by Wehr (1992) for brain warming associated with REM sleep. As demonstrated by Baker et al. (2005), the cerebral cortical EEG during ultradian troughs in BAT and brain temperature is similar to that occurring during slow wave sleep (SWS). Thus the physiological roles of the trough periods may be similar to those proposed for SWS. These include a sleep homeostatic restorative function (Borbely, 1982) and a memory consolidation function (Chrobak and Buzsáki, 1996). The SWS cortical EEG pattern also accompanies torpor and hibernation, states of reduced vigilance and reduced metabolic rate that conserve energy supplies (Heldmaier et al., 2004; Heller and Ruby, 2004). Ultradian troughs in BAT thermogenesis might also serve to conserve bodily fuel supplies. Food restriction can induce torpor with altered BAT metabolism in mice (Swoap et al., 2006) and food restriction led to reduced amplitude of BAT temperature peaks in our rats.

Evidence for ultradian rhythms in metabolism and temperature in primates, including humans

As noted above, it was in humans that REM sleep ultradian rhythmicity, the prototype for the BRAC, was discovered. If the BRAC is present during wakefulness as well as sleep in humans, it must be flexible because culture and contingency play a major role in the patterning of activities during wakefulness. There is no obvious ultradian alertness rhythm in the human EEG during normal waking (Lavie, 1985; LaJambe and Brown, 2008). Nevertheless, in waking primates, a number of physiological and behavioral parameters, including temperature, behavioral activity and plasma levels of noradrenaline in rhesus monkeys, and "basal" metabolic rate in neonatal and adult humans, may exhibit ultradian periodicity of 1–2 h (Bailey et al., 1973; Horne and Whitehead, 1976; Bowden et al., 1978; Levin et al., 1978; Stupfel and Pavely, 1990; Lavie, 1991). It is of great interest that strong evidence for active BAT metabolism has recently been obtained in normal adult humans (Nedergaard et al., 2007; van Marken Lichtenbelt et al., 2009; Saito et al., 2009). An ultradian rhythm in adult human BAT thermogenesis would be of profound physiological significance. Ultradian rhythmicity in brain temperature could elucidate presently unexplained temporal fluctuations in the symptomatology of human neurological and psychiatric disorders.

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