



Multiple ultradian rhythms of metabolism, body temperature and activity in Djungarian hamsters

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Abstract

Djungarian hamsters (*Phodopus sungorus*) living at constant 15 °C T_a in short photoperiod (8:16 h L:D) showed pronounced ultradian rhythms (URs) of metabolic rate (MR), body temperature (T_b) and locomotor activity. The ultradian patterns differed between individuals and varied over time. The period length of URs for MR, T_b and activity was similar although not identical. Wavelet analysis showed that three different URs are existing in parallel, URs of small amplitude and short duration (UR_{small}), URs of medium amplitude and medium duration (UR_{medium}) and URs of large amplitude (UR_{large}), superimposed on each other. UR_{large} were accompanied by an increase in locomotor activity, whereas UR_{small} and UR_{medium} were of metabolic origin with lacking or delayed responses of activity. An energetic challenge to cold which raised total energy requirements by about 50% did not accelerate the period length of URs, but extended the amplitude of UR_{small} and UR_{medium}. UR_{large} corresponds with the URs of activity, feeding and drinking, sleep and arousal as described in previous studies, which are related to midbrain dopaminergic signalling and hypothalamic ultradian signalling. The cause and control of UR_{medium} and UR_{small} is unknown. Their periods are similar to periods of central and peripheral endocrine ultradian signalling, suggesting a link with URs of metabolism.

Keywords Wavelet analysis · Metabolic rate · Bioenergetics · Calorimetry · Infrared thermovision

Abbreviations

CORT	Glucocorticoids and mineralocorticoids
CR	Circadian rhythm
DEE	Daily energy expenditure
DUO	Dopaminergic ultradian oscillator
MR	Metabolic rate ($\text{mLO}_2 \text{ min}^{-1}$)
M_b	Body mass (g)
PVN	Paraventricular nucleus
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
SCN	Suprachiasmatic nucleus
SPZ	Subparaventricular zone
T_b	Body temperature (abdominal)
T_{sf}	Surface temperature

TSH	Thyroid stimulating hormone
TRH	Thyroid releasing hormone
UR	Ultradian rhythm
UR _{small}	Ultradian rhythm with small amplitude
UR _{medium}	Ultradian rhythm with medium amplitude
UR _{large}	Ultradian rhythm with large amplitude

Introduction

Ultradian rhythms (URs) were first discovered in locomotor activity patterns of small mammals (Aschoff and Gerkema 1985; Honma and Hiroshige 1978; Daan and Aschoff 1981). Their real nature was a mystery largely because of their variability and broad frequency range with periods lasting between 20 min and 6 h, quite in contrast to the narrow bandwidth of the circadian rhythms close to 24 h. Research in the past 40 years disclosed that URs are occurring in all animals and even in single cells. They are generated by cellular ultradian oscillators including translation-transcription feedback loops coordinating time dependent cellular processes (Isomura and Kageyama 2014; Goh et al. 2019). URs are coupled between cells, which was recently observed as

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a gap-junction mediated process in confluent fibroblast cell cultures thus allowing coordinated activities of groups of cells or tissues (Yang et al. 2022).

URs are centrally controlled by the hypothalamus suggesting a leading role of the paraventricular nucleus (PVN). In voles, dissections of the PVN suppressed the expression of URs, while dissections of the SCN had no effect on URs but abolished circadian rhythms (CR) (Gerkema et al. 1990b). Ultradian calcium rhythms measured in hypothalamic brain slices in vitro revealed a similar constellation, i.e. calcium URs were generated in the PVN and the subparaventricular zone (SPZ) but not in the SCN (Wu et al. 2018). The latter showed ultradian properties but only when connected with the PVN-SPZ. An additional pathway of central control is linked to the brain dopamine level. In mice the dopamine level in the striatum fluctuates with the ultradian pattern of locomotor activity. A knockout of the dopamine transporter prolongs the ultradian activity period from 4 to 12 h. This suggests the existence of a dopaminergic ultradian oscillator (DUO) for ultradian rhythms of behavioural arousal (Blum et al. 2014; Kashiwagi et al. 2021).

The SCN is considered as the master clock of the circadian rhythm (CR) which establishes internal synchronisation with the circadian oscillators in peripheral cells and tissues as well as external synchronisation with the light dark cycle (reviews in Mohawk et al. 2012; Schibler et al. 2015). Synchronisation with the external light dark signal explains why the circadian clock mechanism has evolved for high precision at a period of 24 h. This need is lacking for URs and explains why they can vary over a broad frequency range from 20 min to 6 h. There is some coordination between the CR and UR (Aschoff and Gerkema 1985, Ono et al. 2015), however URs continue in the absence of external cues as well as following SCN ablation and the concomitant loss of circadian changes in locomotor activity and T_b (Honma and Hiroshige 1978, Gerkema et al. 1990b).

URs are reflecting time dependent and episodic metabolic processes in cells, tissues and the entire organism. Their coordination is essential for metabolic pathway organisation as well as for the amplitude changes of metabolic rate (MR) required for adequate adjustments of energy supply for locomotion, heat production etc. (Ootsuoka et al. 2009, Blessing 2012, Meyer et al. 2012; Grant et al. 2018). There can also be a feedback of environmental conditions on the expression of ultradian rhythms, e.g. food shortage enhances ultradian rhythms in voles (van Rosmalen and Hut 2021).

URs of animals were demonstrated mostly for activity behaviour, feeding, neural and endocrine rhythms. Little attention was paid to URs of metabolic rate and other metabolic parameters, largely because of technical limitations for measuring animal metabolism in vivo at high resolution over prolonged periods of time. Brodsky (2014) has emphasized that circadian (ultradian) rhythms of metabolic processes

like protein biosynthesis, cell respiration and the mitotic cell cycle occur in parallel with ultradian rhythms of endocrines, suggesting that endocrine signalling plays an important role in the control of cellular ultradian rhythms. Hormones which are known for direct effects on metabolism of whole animals, like catecholamines, thyroid hormones and glucocorticoids, may therefore also play an important role in the expression of ultradian metabolic processes on the whole animal level.

We recorded metabolic rate and respiratory exchange rate of Djungarian hamsters at 1 min intervals continuously over several months in parallel with telemetry of T_b and locomotor activity to obtain a detailed picture of stability and changes of URs in all four parameters, and to analyse the role of ultradian rhythms for the control of metabolic rate.

Methods

Animals

Djungarian Hamsters (*Phodopus sungorus*) were bred and raised at the Department of Biology at Marburg University, as described previously (Ruf et al. 1993). They received food (Sniff V2140-000) and water ad libitum. At the age of three months they were kept singly in standard Makrolon cages, Type 3, and transferred from long photoperiod (L:D 16:8 h) and 23 °C T_a to short photoperiod (L:D 8:16 h) at 23 °C T_a . Body mass and fur colour index were recorded at weekly intervals to follow acclimation to short photoperiod.

Fifteen hamsters of both sexes were used in this study. They were kept in metabolic cages for two months for continuous records of MR (see also Heldmaier et al. 2024). Eight of these hamsters were additionally implanted with transmitters for T_b and locomotor activity for the simultaneous measurement of MR, T_b , and activity and the analysis of ultradian rhythms. Seven of these hamsters received transmitters for T_b only and were used for the energy challenge experiments. Description of methods see below.

For measurement of MR the hamsters were transferred to ventilated cages (Zoonlab, Castrop-Rauxel, Germany, IVC cages, Type 1 long (volume 8 L)), with little bedding material (wood shavings 80 g) and two paper towels, which they gnawed to build a nest. One separate cage setup (glass terrarium, volume 12 L) was equipped with a video camera and an IR-thermovision camera (Optris PI450, Optris GmbH, Berlin) for additional continuous observation of hamster behaviour and measurement of surface temperature. Ambient temperature of the climate chamber was controlled at 14 ± 1 °C and was recorded for each hamster by a thermocouple inside the cage. Cages were cleaned and bedding material renewed at weekly intervals. For the latter measurements 4 out of the 8 hamsters were transferred for at least 10 days to the glass terrarium and then returned to their IVC cage.

Transmitters for T_b and locomotor activity

Transmitters for simultaneous recording of T_b and activity (Vitalview 4000, Starr Life Sciences, Oakmont, PA, USA) were implanted with the same procedure as described previously for other abdominal transmitters (e.g. Bräulke et al. 2008). Briefly, the hamsters were initially anaesthetized with Rompun (1 mg kg⁻¹) and Ketanest (50 mg kg⁻¹). Rompun was used as an analgesic. During surgery anaesthesia was controlled and maintained with Isoflurane. The transmitter was implanted into the abdominal cavity without fixation. The wound was closed with an abdominal suture plus a skin suture. Typically, the hamsters aroused from anaesthesia within 20 min, walked around and started drinking and feeding, and were left for 5 days in their cage for full recovery. T_b (°C) and activity (counts min⁻¹) was recorded in 1 min intervals during the entire measurement sessions lasting up to three months.

Behaviour and locomotion by infrared thermovision

Behaviour and locomotor activity were further analysed by an infrared camera system (Optris PI 450i, Optris GmbH, Berlin) which was mounted on the top of the cage. This auto-calibrated thermovision camera measured surface temperatures (T_{sf}) with a spatial resolution of 382 × 288 pixels and a thermal resolution of 0.04 K. T_{sf} images were continuously recorded and stored at 1 s intervals. For analysis we specified three measurement areas in the T_{sf} images. One area traced the hamster, following it at any position in the cage, within its nest or outside the nest, during feeding and drinking. Two fixed measurement areas monitored the bottom of the cage outside the nest. This allowed us to locate the position of the hamster, its T_{sf} and the surrounding temperatures. Hamster T_{sf} 's were always higher than the cage bottom, and T_{sf} of eyes and nostrils were close to core temperature of the hamster. Body movements caused short term changes of T_{sf} . Standard deviation (SD) of T_{sf} min⁻¹ was calculated as an index for total activity of the hamsters.

Metabolic rate

Metabolic rate (mL O₂ min⁻¹) was measured with CaloBoxes (Phenosys GmbH, Berlin, Germany) which were directly connected to the lids of ventilated cages (Zoonlab GmbH, IVC type 1 long, Castrop-Rauxel, Germany) (Elfers et al. 2022). We used up to 8 CaloBoxes simultaneously to obtain continuous records of individual hamsters. The CaloBoxes were placed near the animal cages in the climate chamber. Sample air was drawn from the animal cage with flow rates of ~85 L/h. Ambient air of the climate chamber was used as reference air. To buffer short term variations of climate chamber air conditions air supply for the animal

cages and reference air were drawn from a 100 L reservoir (dust bin) inside the climate chamber.

The CaloBox measured O₂, CO₂, and water vapor content every 4 s and calculated RER and heat production. Heat production (HP) was calculated by using the equation $HP[mW] = (4.44 + 1.43 \cdot RER) \cdot [mLO_2 h^{-1}]$ which provides the oxidative energy retrieved from mixed combustion of carbohydrates and lipids (Heldmaier 1975). Zero adjustment and O₂-sensor calibration with reference air occurred every 15 min. The CaloBox did not need any air processing (drying, CO₂ absorption) prior to analysis but measured the composition of incoming air. Results were collected and stored every 30 s or 60 s (further details see Elfers et al. 2022; Wellbrock et al. 2022).

Response of ultradian rhythms to thermal challenge

To measure the effect of energetic challenges on URs, data from a previous study were reanalysed. In this study MR was measured with a different system using an O₂ analyser (S3a, Applied Electrochemistry), and a CO₂ analyser (Maihak) (method see Bräulke et al. 2008, 2010). Air was dried with a freeze trap. Data were sampled at 3 or 4 min intervals.

Identification of ultradian rhythms

Long-term simultaneous records of T_b , activity, and metabolic rate were used to identify ultradian rhythms. Ultradian rhythms are rather variable unlike the stable circadian rhythms which are controlled by an endogenous clock. To identify regular structures in ultradian rhythms we performed a Wavelet analysis and calculated the power spectrum (R package WaveletComp (Roesch and Schmidbauer 2018)) in R 4.2.2 (R Core Team 2022)) of regular, individual records of metabolic rate, T_b and locomotor activity. The wavelet transform is a method for determining instantaneous periods with good resolution in both time and frequency. Unlike Fourier transformations, wavelets are not based on infinitive trigonometric functions but on short waves that decay quickly to zero. This is also true for the popular Morlet wavelet, a complex sine decaying on both sides of the centre, which is the underlying function used by WaveletComp. The principal operation performed is the local convolution of the stretched or squished wavelets along the time series, effectively computing similarities. Thus, wavelet transformation analyses localized variations of amplitude within a time series. The amplitude of ultradian rhythms was determined by searching for peak maxima using a search window adjusted to the ultradian periods retrieved for each hamster and each day. To make URs highly comparable we did not change the wavelet or its parameters for the analysis of different variables (i.e., T_b , MR, activity). For the same reason we used continuous wavelet transform for all variables, despite certain differences between discrete and continuous data (Leise et al. 2013).

In the R package WaveletComp the statistical significance of the power of peaks (amplitudes relative to noise) is computed by simulation (we used 150), i.e., the comparison of actual power with random noise at the same frequency. Here we called the function with method="shuffle". This randomly shuffles the original data instead of using white noise, to make sure that the mode of data sampling does not affect results (Nemec and Nemec 1985). The frequencies inspected here were in the ultradian range (0.3–6 h). Because we were not interested in circadian rhythms, we limited data to 24 h. Time series analysed were also restricted to 24 h to avoid mixing days with and without torpor (for general recommendations on data length see Leise and Harrington 2011). Time series were on a regular grid ($n = 1440$) at 1 min intervals.

For wavelet analysis output-graphs the number of colours was set to 250, and power ridges (upper bands of neighbouring values in the frequency domain) were plotted but not further considered. To further determine the highest powers in a periodogram we used function findpeaks in the package pracma (Borchers 2019) using default parameters.

We mainly preferred wavelet analysis over traditional Fourier and related MESA or Lomb-Scargle periodograms because they all have potentially large peaks at harmonics of 24 h (12 h, 8 h, 6 h, etc.), even when the measured signal involves no ultradian periods. Data folding methods such as the Chi^2 periodogram repeat every short-term peak at its multiple (Ruf 1999). These traditional methods will not be useful in measuring the true period(s) of ultradian activity/TB/MR patterns (Leise 2013). More appropriate are time–frequency

methods, such as wavelet analysis “that can localize frequency estimates in time are more appropriate for analysis of ultradian periods and of fluctuations in the period” (Leise 2013).

Statistical evaluation of results

Results are either presented as original data or mean values. For comparison between groups ANOVA was used with $p < 0.05$ as general level of significance. Due to the nature of data in most cases ANOVA of repeated measures was applied, and differences between groups were compared with the Holm-Sidak Method (Jandel SigmaPlot10). Non-parametric tests (Tukey test, Wilcoxon test) were applied for data with a non-normal distribution. Visual inspection of model residuals (qqnorm plots) in R, gave no reason to suspect a notable deviation from normality.

Results

Ultradian rhythms of metabolic rate, activity and body temperature

24 h records of metabolic rate, body temperature and locomotor activity of Djungarian hamsters kept in constant conditions were characterized by the presence of ultradian variations. The pattern varied between individuals but each hamster showed an apparently synchronous change

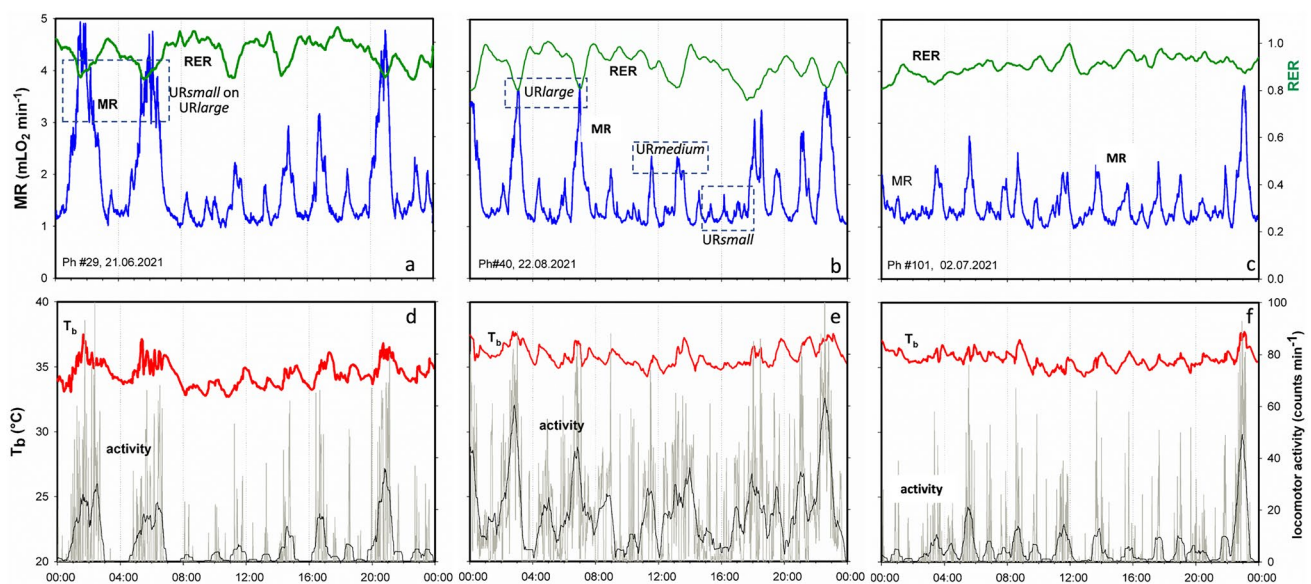


Fig. 1 Ultradian rhythms of MR, RER, T_b and locomotor activity of short day acclimated Djungarian hamsters. Records of three different individuals and days are shown as examples for the individual variability of ultradian behaviour. Ph#29, male, M_b 33.9 g; Ph#40, female, M_b 32.5 g; Ph#101, male, M_b 31.2 g. Data were obtained in 1 min

intervals. For locomotor activity raw data (gray) and 30 min running average (black) are shown. RER is presented as 30 min running average of raw data. URs of MR are characterized by at least three different amplitudes, i.e. URsmall, URmedium and URLarge

in MR, T_b and bursts of locomotor activity (Fig. 1). The original record of MR revealed the existence of at least three different URs, which are existing simultaneously (Fig. 1). Most obvious were large amplitude fluctuations, where MR was $> 100\%$ above RMR (*URlarge*). Small amplitude fluctuations about 20% above RMR were traceable throughout the entire day and are also found superimposed as peaks on *URlarge*. In between these two extreme fluctuations of MR a third category of medium sized URs could be discriminated with an amplitude of about 50% above RMR (*URmedium*). Examples for these three different URs are framed in Fig. 1b, as well as superimposition of *URsmall* on *URlarge* shown in Fig. 1a. The 24hourly records of T_b and locomotor activity show URs with rather uniform amplitudes (Fig. 1 d, e, f) as compared to the varying amplitude of MR URs (Fig. 1a, b, c).

Wavelet analysis of MR revealed three different periodicities which can be associated with three URs of different amplitude. The original records showed *URsmall* with

a period of about 1 h (0.98, 1.5, 1.1 h in the three examples in Fig. 2), *URmedium* had periods of about 2 h (2.07, 2.17, 1.33 h in Fig. 2), and *URlarge* periods of about 3 h (3.91, 3.65, 1.91 in Fig. 2). These examples demonstrate the individual variability of period length of all three URs.

Wavelet analysis of the 24hourly records of T_b and activity revealed that they also contained three different ultradian periodicities although these were not apparent in the original records (Figs. 1 and 2) Their period lengths were comparable to the periods retrieved from wavelet analysis of MR URs, e.g. hamster Ph#29 (Fig. 2) had a *URsmall* period of 0.98 h for MR, 0.51 h for activity and 0.77 for T_b . The corresponding values for *URlarge* were 3.91 h, 3.37 h and 4.59 h.

Within each individual the ultradian rhythms of MR, T_b and locomotor activity were similar but not identical, e.g. *URsmall* of hamster Ph#29 had a period of 0.98 h for MR, 0.51 h for activity and 0.77 h for T_b . Corresponding periods for *URmedium* were 2.07 for MR, 2.07 for

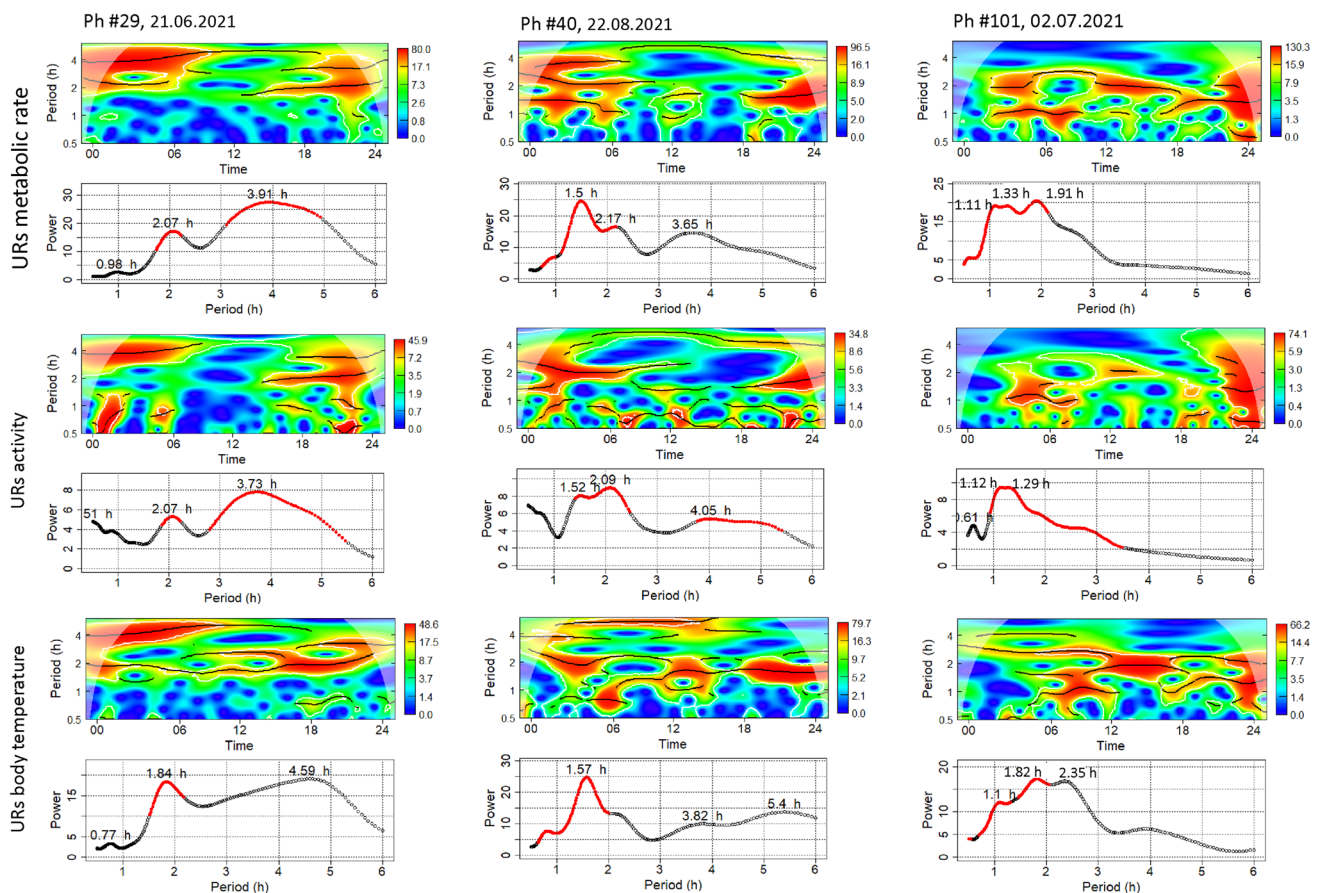


Fig. 2 Wavelet analysis and power spectrum of ultradian periodicity of MR (top rows of graphs), locomotor activity (middle row of graphs) and T_b (bottom row of graphs) of the three 24 hourly records presented in Fig. 1. Each graph has two parts with a heatmap of the wavelet analysis on top and the periodogram at the bottom. Red

areas in the heatmap indicate the presence of significant periodicity ($p < 0.05$). Red sections of the lines indicate significant ($\alpha = 0.01$) periods as determined from random shuffles of original values of MR, activity and T_b

activity and 1.84 h for T_b . For *URlarge* the periods for MR, activity and T_b varied internally from 3.91, 3.73, and 4.59 h. (Fig. 2). Wavelet analysis showed additionally that the period length of ultradian rhythms was not constant throughout the day but varied, as well as there were changes in amplitude with lowest values observed during the diurnal resting phase of the night active Djungarian hamsters (Fig. 1).

Long term variability of ultradian rhythms

The ultradian pattern of individual hamsters varied from day to day (Fig. 3), although the hamsters were kept in a constant environment with ad libitum food and water supply, suggesting an endogenous origin of these variations. Wavelet analysis was performed for each single 24hourly record revealed almost daily changes of ultradian periods for URs of MR, activity as well as T_b . Many of these changes occurred in parallel in *URsmall*, *URmedium* and *URlarge*. The time course of UR periods revealed a pattern where groups of days with relatively short ultradian periods alternated with groups of days with long ultradian periods, suggesting infradian influences on ultradian periodicity.

The apparent synchrony between *URsmall*, *URmedium* and *URlarge* was analysed by pairwise comparisons of the time course of UR periods. *URsmall* versus *URmedium*,

URsmall versus *URlarge*, and *URmedium* versus *URlarge*, as shown in the six graphs from 2 hamsters in Fig. 4. If the three different UR categories would be harmonics of one ultradian oscillator one would expect straight linear regressions and correlations of periods between UR categories. The scattered data points do not support this view. In five of the six comparisons UR periods were significantly correlated, i.e. they are in synchrony with each other but the scattered data points doubt that they operate as fractions of each other. In one hamster the MR periods of *URsmall* versus *URlarge* were even lacking evidence for correlation.

This interaction between ultradian periods of MR, activity and T_b was evaluated in 8 hamsters. Individual 24hourly records were obtained from recordings which lasted between 14 and 24 days. 72 pairwise comparisons revealed 47 significant correlations ($p < 0.05$), i.e. the daily changes of UR periods of MR, activity and T_b over time were synchronized in 65.3% of all cases. Best synchronisation was found between *URsmall*, *URmedium* and *URlarge* of MR ($p < 0.05$ in 20 of 24 pairwise comparisons), whereas URs of activity were correlated only in 13 out of 24 cases, and the time course of UR periods for T_b was significant in 14 out of 24 pairwise comparisons ($p < 0.05$). This suggests the existence of more than one cellular or central oscillator for ultradian rhythms.

Fig. 3 Development of UR period duration in two hamsters over the course of 21 days (Ph#44, male, mean M_b 31.5 g) and 24 days (Ph#66, female, mean M_b 33.6 g). The amplitude and frequency indicate three different ultradian rhythms for metabolic rate, locomotor activity as well as body temperature, filled square *URlarge* ultradian large amplitude, unfilled circle *URmedium* ultradian medium amplitude, filled circle *URsmall* ultradian small amplitude

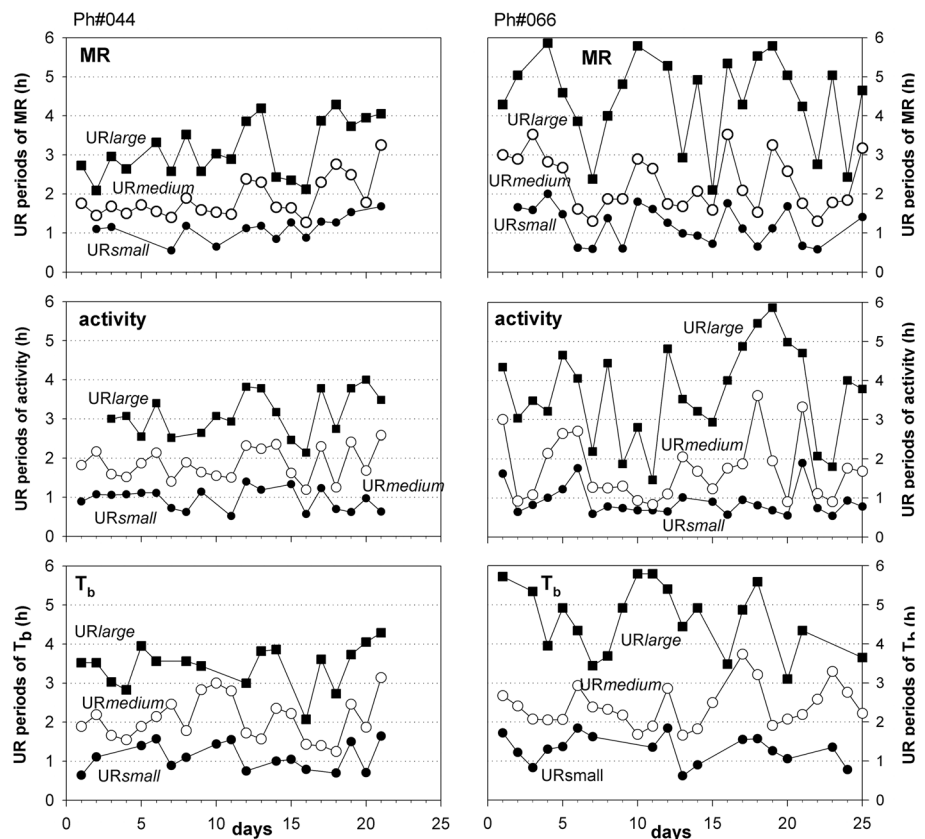


Fig. 4 Correlations between the three ultradian periods UR_{small} versus UR_{large} , UR_{small} versus UR_{medium} , and UR_{medium} versus UR_{large} obtained from records of metabolic rate over the course of 21 days (Ph#44) and 25 days (Ph#66). Time course of data see Fig. 3

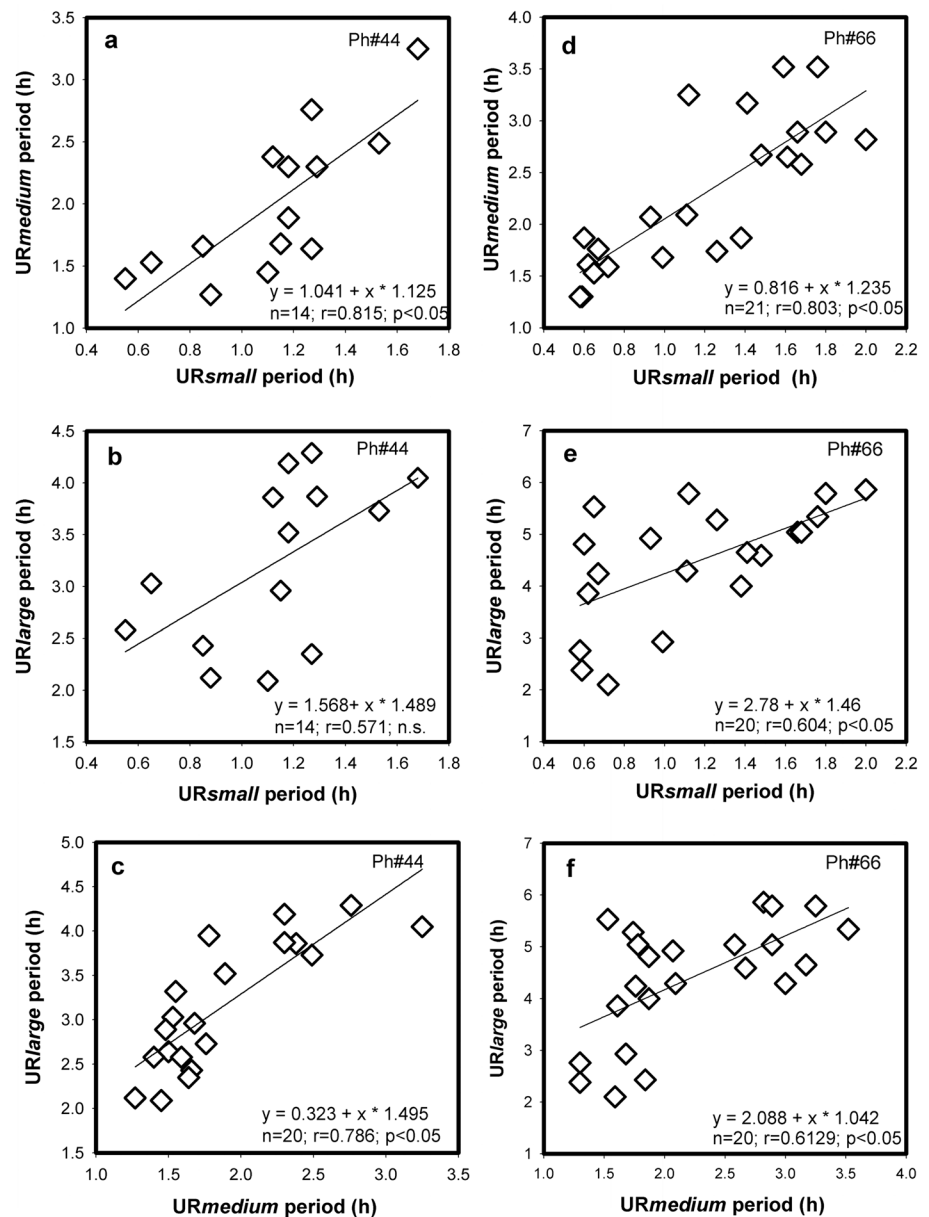


Table 1 Properties of UR_{small} , UR_{medium} and UR_{large} of metabolic rate (MR), and interaction with URs of T_b and locomotor activity. URs were determined in 24hourly records of $n=8$ hamsters by wavelet analysis. The individual period lengths of these URs were used to identify single ultradian bursts, their minimum and peak

for MR, T_b and activity. For statistical analysis one way ANOVA repeated measures for three groups and pairwise multiple comparison with the Holm-Sidak method were used. Means $\pm$ SEM. Results from activity amplitude failed normal distribution and therefore the Tukey method was used

	UR_{small}	UR_{medium}	UR_{large}	
n hamsters, N bursts	$n=8$, $N=79$	$n=8$, $N=57$	$n=8$, $N=64$	
Ultradian bursts per day	$>9.8 \pm 3.6^*$	$>7.1 \pm 1.8^*$	8.0 ± 1.9	
Period duration [min]	74.9 ± 9.11	132.9 ± 14.2	203.2 ± 22.2	$p < 0.01$
Peak MR [$\text{mL O}_2 \text{ min}^{-1}$]	1.375 ± 0.039	1.780 ± 0.084	3.170 ± 0.131	$p < 0.01$
MR amplitude [$\text{mL O}_2 \text{ min}^{-1}$]	0.231 ± 0.023	0.520 ± 0.033	1.915 ± 0.147	$p < 0.05$
T_b amplitude [$^{\circ}\text{C}$]	-0.04 ± 0.05	0.29 ± 0.06	1.12 ± 0.14	$p < 0.05$
Activity amplitude [I min^{-1}]	1.19 ± 0.42	0.301 ± 0.06	15.47 ± 1.08	Small vs medium = n.s., other pairs $p < 0.05$

*Only bursts close to baseline level were considered. The true number of UR_{small} and UR_{medium} may be greater due of overlap with UR_{large}

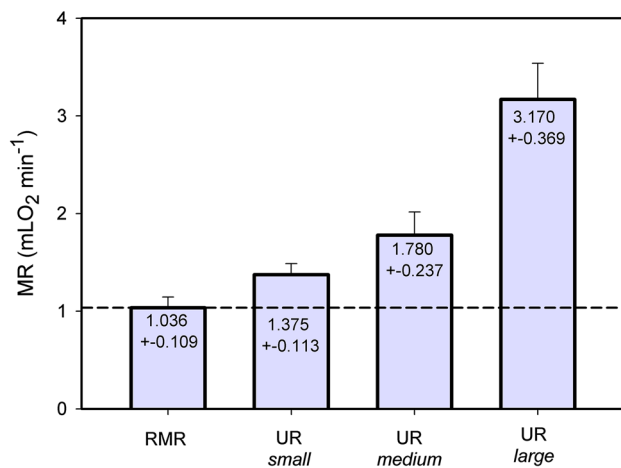


Fig. 5 Maximum MR during URs. The level of RMR and the amplitude of UR_{small}, UR_{medium} and UR_{large} were calculated on the basis of individual periodograms and wavelet analysis for at least 14 days. Mean values $\pm$ SD of 8 hamsters. Differences between RMR, UR_{small}, UR_{medium} and UR_{large} were significant with $p < 0.001$ ($n = 8$; one way ANOVA for repeated measures, pairwise comparisons by Holm-Sidak method)

Amplitude of ultradian rhythms

The results of wavelet analysis were used to determine the amplitude of ultradian bursts and to analyse further properties which differentiate between UR_{small}, UR_{medium} and UR_{large} (Table 1). The amplitude of URs was determined by searching the peak MRs in each 24hourly record with search windows adapted to the periods of UR_{small}, UR_{medium} and UR_{large} for this particular 24 h record. Following peak identification a similar procedure was used to identify the minimum MR prior to the peak, which allowed to calculate the amplitude of changes in MR, activity and T_b during ultradian bursts (Table 1 and Fig. 5). The three UR categories showed peak values of MR, T_b and activity with three different magnitudes as well as three different amplitudes of

change. This supported the initial discrimination of ultradian bursts into the categories small, medium and large.

The original records of MR, activity and T_b (Figs. 1, 2) suggest a lower amplitude or lower intensity of URs during daytime hours (08:00...16:00) i.e. the circadian resting phase of Djungarian hamsters. To quantify this diurnal regression UR_{large} periods (Fig. 1) were separated into daytime values and nighttime values (Table 2). The peak MR, as well as the amplitude of MR, T_b and activity were significantly reduced during daytime hours suggesting an attenuation of UR_{large} during the resting phase by about 40%.

The time course of UR periods (Fig. 3) suggests that calculation of mean values for UR periods, as well as the statistical discrimination between URs of MR, and activity and T_b , requires prolonged recordings to compensate for infradian influences on ultradian periods. Therefore, individual means for the period duration of UR_{small}, UR_{medium} and UR_{large} were calculated for MR, activity and T_b from 8 hamsters recorded for 14 and 24 days without interruption. This calculation revealed significant differences between UR_{small}, UR_{medium} and UR_{large} for MR, T_b and activity (Fig. 6). When comparing the corresponding UR periods within UR_{small}, UR_{medium} and UR_{large} the periods for T_b were significantly longer than the corresponding UR periods of activity in all three UR categories. In UR_{large} the period for T_b was also longer than the corresponding MR period. At present it is not clear if this indicates separate ultradian oscillators or if it is a side action of thermal inertia in contrast to the immediacy of activity and metabolic responses.

Dynamics of ultradian bursts

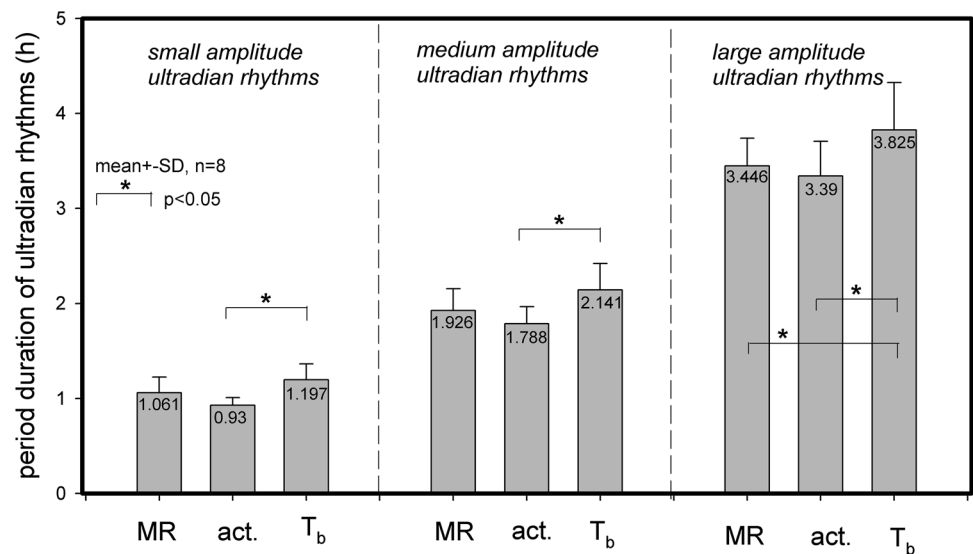
URs of large amplitude (UR_{large}) were starting with a simultaneous increase of locomotor activity, metabolic rate and body temperature, and the RER was gradually decreasing, as shown for three different examples in Fig. 7.

Table 2 Ultradian bursts during the circadian activity phase versus the resting phase. The results are from the same hamsters as described in Table 1. Hamsters are nocturnal. The light phase from 08:00...16:00 was considered as their resting phase. UR_{large} bursts occurring during the resting phase were compared with UR_{large}

bursts during the activity phase (00:00.....07:59 and 16:01.....24:00). Amplitudes are the difference between minimum and maximum MR, T_b and activity of each burst. Mean $\pm$ SEM. Paired t-test. Results for the T_b amplitude were not normally distributed and therefore compared using a Wilcoxon test

	Resting phase	Activity phase	Significance	Reduction during resting phase
n hamsters, N UR _{large} bursts	n = 8, N = 22	n = 8, N = 42		
peak MR [mLO ₂ min ⁻¹]	2.31 $\pm$ 0.17	3.24 $\pm$ 0.17	$p = 0.002$	28.7%
MR amplitude [mLO ₂ min ⁻¹]	1.35 $\pm$ 0.18	2.18 $\pm$ 0.16	$p = 0.003$	38.1%
T_b amplitude [°C]	0.80 $\pm$ 0.10	1.37 $\pm$ 0.16	$p = 0.008$	0.57 °C
Activity amplitude [I min ⁻¹]	7.51 $\pm$ 1.42	18.74 $\pm$ 1.41	$p = 0.002$	59.5%

Fig. 6 Mean values $\pm$ SD of ultradian period durations for MR (VO_2), locomotor activity (act) and body temperature (T_b) from 8 hamsters. SD represents the variation between individuals. Data were obtained from similar evaluations as presented for two hamsters in Fig. 3. Data with in *URsmall*, *URmedium* and *URLarge* were compared by repeated measures ANOVA. Differences between group specific periods were analysed by the Holm-Sidak Method and significant ($p < 0.05$) differences were marked by connecting bars



The large amplitude bursts lasted about 1–2 h and each parameter returned to its initial level. The return differed between the three physiological parameters, especially T_b remained elevated for an extended period of time.

URs of small (*URsmall*) and medium (*URmedium*) amplitude frequently showed a different pattern, with a predominant initial increase of metabolic rate, and lacking or delayed changes in locomotor activity and T_b (Fig. 8). During 68% of *URsmall* metabolic bursts (79 bursts from 8 hamsters) the increase of MR was not accompanied by an increase of T_b and 44% lacked an increase in locomotor activity, although MR was elevated by about 20%, during an initial rise time of MR from minimum to peak MR which lasted 13.12 ± 3.60 min. This explains the negative mean values of T_b amplitude and the minor changes of activity for *URsmall* in Table 1. Metabolic bursts of *URmedium* were lacking an increase of locomotor activity in 21% of 57 cases analysed, whereas in *URLarge* the increase of MR was always accompanied by a simultaneous increase in locomotor activity and T_b as shown in Fig. 8.

Large amplitude bursts were also characterized by a decrease of RER as summarized in Fig. 9, indicating enhanced lipid oxidation during *URLarge*. An increase of mean MR from 1.03 to $3.47 \text{ mL O}_2 \text{ min}^{-1}$ during *URLarge*, was paralleled by a reduction of RER from 0.96 to 0.84. The hamsters were fed ad libitum with pellet food (breeding diet with high protein content) and burned primarily carbohydrates at the resting level of MR as indicated by the RER of 0.96. The reduction of the RER to 0.84 during the 3.38fold increase of MR indicates a major rerouting of metabolism to combustion of lipids. This occurred without any external stimulation indicating that metabolic fuel processing spontaneously altered between carbohydrate and lipid metabolism due to the activity of URs. In few cases we observed no reduction of RER during *URLarge* which might have been

caused by simultaneous food uptake, absorption and metabolism of carbohydrates in the pellet diet.

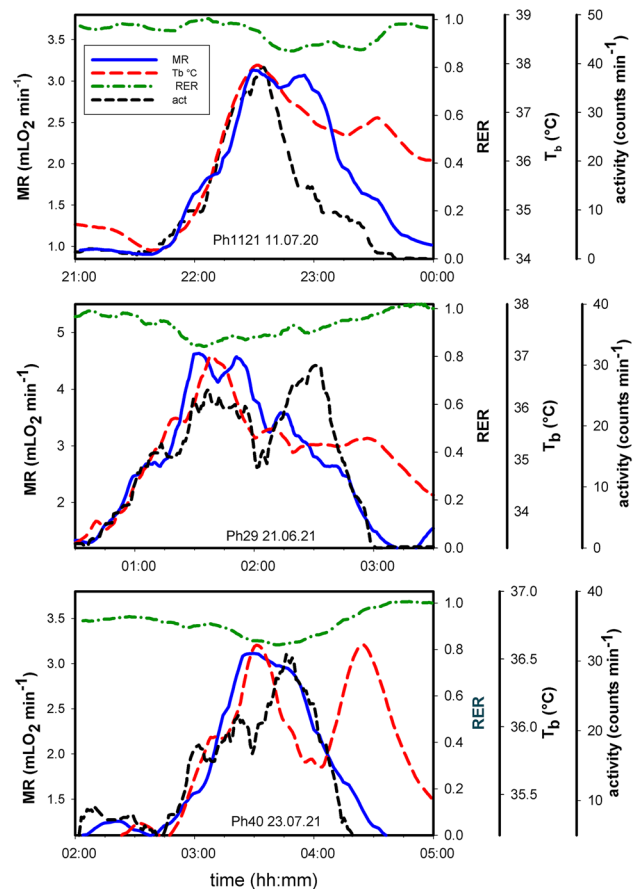


Fig. 7 *URLarge* amplitude dynamics. Three examples of changes in metabolic rate, RER, body temperature and locomotor activity during ultradian large amplitude bursts. Data for MR, T_b and act were scaled to similar amplitude to facilitate the comparison of changes during individual ultradian bursts

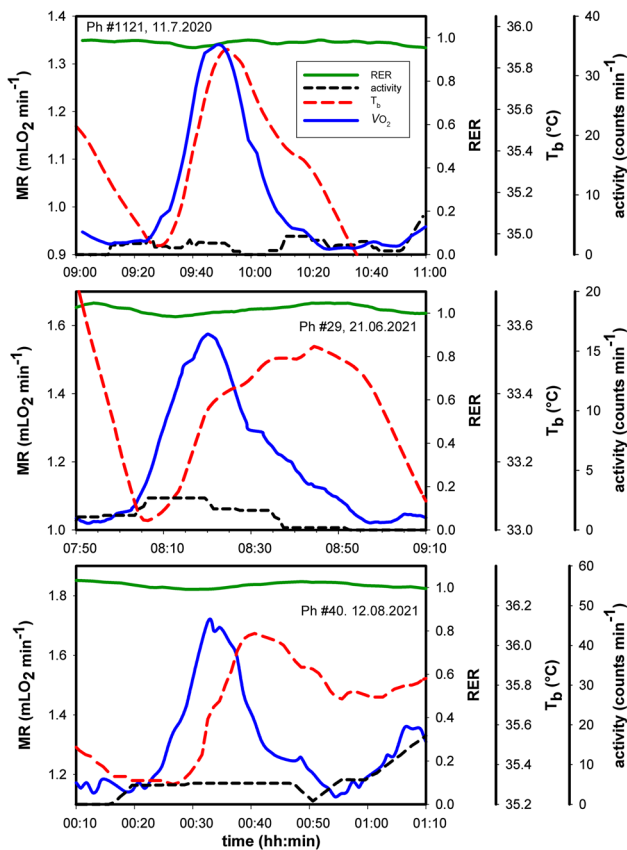


Fig. 8 UR_{small} amplitude dynamics. Three examples of changes in metabolic rate, RER, body temperature and locomotor activity during ultradian small amplitude bursts. Further details see legend of Fig. 8

Ultradian activity pattern and energy balance

The total amount of energy expenses for ultradian variations of MR was estimated from the difference between total energy expenses per day (DEE) and the energy expenses for RMR. The DEE of hamsters at 15 °C T_a was 44.4 ± 4.0 kJ day⁻¹ and their RMR was 29.8 ± 2.7 kJ day⁻¹ ($n=8$, M_b 31.7 ± 2.5 g). This means that the ultradian system required 14.6 ± 3.1 kJ day⁻¹, i.e. 32.7% of the daily energy budget at a T_a of 15 °C (calculations are based on the data used for Fig. 5).

Activity of hamsters was recorded with implanted transmitters. They responded to any movement of the body and may not have mirrored the energy required for any kind of activity. To obtain further details about activity we additionally recorded the position and movements of hamsters with infrared thermovision which allowed to trace the posture and position of the hamster in the cage and allowed to discriminate if hamsters were active inside or outside their nest. Both methods were applied simultaneously to measurements of MR and T_b (Fig. 10). Telemetry and MR was recorded in 1 min intervals, IR images were recorded in 1 s intervals.

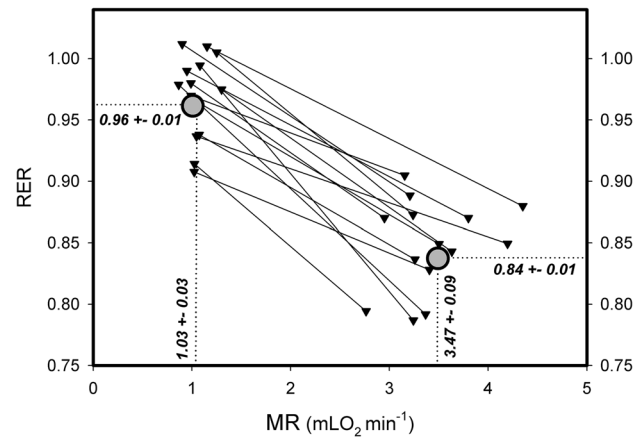


Fig. 9 RER during large amplitude bursts of metabolic rate. MR and RER before an ultradian burst are compared with maximum metabolic rate and the associated minimum RER. 14 large amplitude variations were analysed from 8 different hamsters. Mean values were calculated for 8 hamsters

To automatize the evaluation of IR images we defined three areas, i.e. one area which traced the hamster and 2 stable areas representing the cage area outside the nest. Maximum body T_{sf} of the hamster varied between 35.5 °C, i.e. close to core T_b , and 15 °C close to T_{sf} of the cage bottom, depending upon the posture towards the thermovision camera (area 1 in Fig. 7a, and red line in Fig. 7b). Activity was calculated from the amplitude of T_{sf} changes (Fig. 7b black line), i.e. the standard deviation of T_{sf} min⁻¹. This estimate of activity closely parallels the activity pattern obtained with implanted transmitter (Fig. 7e) and the ultradian pattern of MR (Fig. 7d).

The 7 UR_{large} bursts of this hamster during 24 h were always associated with activity outside the nest. During the diurnal resting phase the hamster was less active outside the nest. In total it spent 195 min outside the nest for feeding, drinking, defecation and other activities (mean 177 ± 25 min in four hamsters). Thus, the hamsters remained in the nest for ~21 h per day, where they were active too with small movements, grooming and nest building. A motionless rest in the nest was rarely seen and limited to short periods of a few seconds or minutes. This can be concluded from the amplitude of hamster T_{sf} which was > 1 °C most of the time. In a motionless hamster one would expect T_{sf} changes < 0.5 °C sec⁻¹, similar to stable readings of cage bottom T_{sf} which varied < 0.5 °C sec⁻¹.

Activity records with implanted transmitters and activity records with the thermovision camera were closely correlated (Fig. 11a). Both methods had similar slopes for the relation between an increase in activity and an increase of energy requirements (Fig. 11 b, c), underlining the close relation between locomotor activity and gross ultradian changes of MR. The comparison of both methods revealed

that infrared thermovision is a suitable method for the measurement of total activity in small rodents without requiring surgery, and provides additional information on location and behaviour of an animal its T_{sf} as well as core temperature.

Response of URs to energetic challenge

Cold exposure will increase energy expenditure to maintain euthermy. It is unknown if this changes the periodicity or amplitude of ultradian metabolic processes. Short day acclimated hamsters were kept at 24 °C T_a (thermoneutral) or at 16 °C T_a (moderate cold exposure) in type 1 cages with a small amount of wood shavings as bedding material. MR and T_b was recorded continuously. The hamsters displayed ultradian variations of MR (see individual examples in Fig. 12). The RMR of hamsters was tracked by the minima of ultradian metabolic bursts, and a 24hourly mean of RMR was calculated. This was subtracted from the total metabolic rate per day (daily energy expenditure, DEE) to obtain the contribution of ultradian metabolic bursts. Moderate cold exposure by changing T_a from 24 to 16 °C increased DEE of hamsters from 34.8 kJ day⁻¹ to 58.1 kJ day⁻¹, as expected (Table 3, Fig. 12a, b, c). This was largely due to an increase of RMR from 0.845 mLO₂ min⁻¹ to 1.432 mLO₂ min⁻¹.

The energy requirements of ultradian metabolic variations were 0.511 and 0.409 mLO₂ min⁻¹ at 24 °C and 16 °C T_a , respectively (difference not significant). Thus, surprisingly the energy expenditure of URs remained constant at both temperatures. It was only the rise of RMR which could be held responsible for the increase of total energy requirements in moderate cold. Due to the rise in RMR the relative amount of ultradian energy expenses decreased from 37.9% of DEE at thermoneutrality to 22.4% of DEE in moderate cold (Table 3). The periods of UR_{small}, UR_{medium} and UR_{large} as well as their pattern was similar in both groups (Table 3, Fig. 12a, b and c).

URs are tightly linked with behavioural activity of animals. We stimulated behavioural activity of hamsters at 16 °C by offering them nesting material (cellulose bulk). The presence of a nest reduced MR but increased the incidence of spontaneous daily torpor in hamsters. Therefore, we compared only 12 h records during the nocturnal euthermy phase (Table 4, Fig. 12d, e, f). Three of the hamsters included in the previous group at 16 °C T_a received nesting material and immediately built a well insulating nest which reduced total MR from 1.822 to 1.092 mLO₂ min⁻¹. The period of URs as well as the metabolic expenses of UR MR remained unchanged, similar to the temperature effect described in Table 3.

However, the pattern of URs changed with the presence of a nest. URs were dominated by a few large peaks of MR (Fig. 12). This reduced the average peak MR from 2.124 mLO₂ min⁻¹ to 1.624 mLO₂ min⁻¹ whereby the total amount

of MR UR remained unchanged (Table 2). The visual impression of a different pattern is underlined by counting the number of ultradian MR peaks > 2.0 mLO₂ min⁻¹. This arbitrary threshold revealed 4 peaks per 12 h in hamsters using a nest instead of 10 peaks per 12 h in hamsters living without a nest.

Discussion

Multiple ultradian rhythms

Djungarian hamsters showed similar URs in metabolic rate (MR), locomotor activity and T_b . MR revealed the most detailed insights into the complexity of ultradian organisation of body functions. At least three different URs were running in parallel with different amplitudes and variable frequencies. They could be differentiated by amplitude and period. Rhythms of small (UR_{small}), medium (UR_{medium}) and large (UR_{large}) amplitude were associated with short (~ 1 h), medium (~ 1.5–2.2 h) and large (~ 2.0–5.0 h) periods, respectively. These were running in parallel in MR, T_b , as well as activity. This differs from previous studies in Djungarian hamsters and other small mammals where always one systemic UR was described with a period ranging between 1 and 6 h (Prendergast and Zucker 2012, Gerkema et al. 1990b, Honma and Hiroshige 1978).

The metabolic ultradian system required a substantial amount of the total daily energy budget. Most hamsters required a constant amount of 14.6 kJ day⁻¹ for their MR URs, i.e. up to 30% of the total daily energy requirements. Moderate cold exposure as well as the lack of a nest enhanced the MR for heat production to maintain euthermy. This was solely achieved by an increase of RMR, whereas ultradian MR remained unchanged at the thermal challenges presently used. Previous studies in Djungarian hamsters have shown that the difference between DEE and RMR increases in severe cold (– 1 °C through – 15 °C T_a) suggesting an even greater cost of activity during URs (Ruf and Graf 2010).

The discovery of different URs may partly be explained by technical reasons and the high resolution of our recording device for MR. We recorded MR, T_b and activity at 30 s or 1 min sampling intervals continuously over several months. Previous long-term studies on ultradian rhythms used longer sampling intervals for food intake, wheel running or locomotor activity by passive IR detectors which required whole body displacement (Gerkema et al. 1990a, Gerkema et al. 1991, Prendergast and Zucker 2012, Ono et al. 2015). URs of endocrines depend upon sampling of body fluids which are only accessible at larger intervals of 10, 20 or 30 min., which can only be continued for a limited period of time. This limits the resolution for differentiation of URs and focusses interpretation on one unique UR. Our

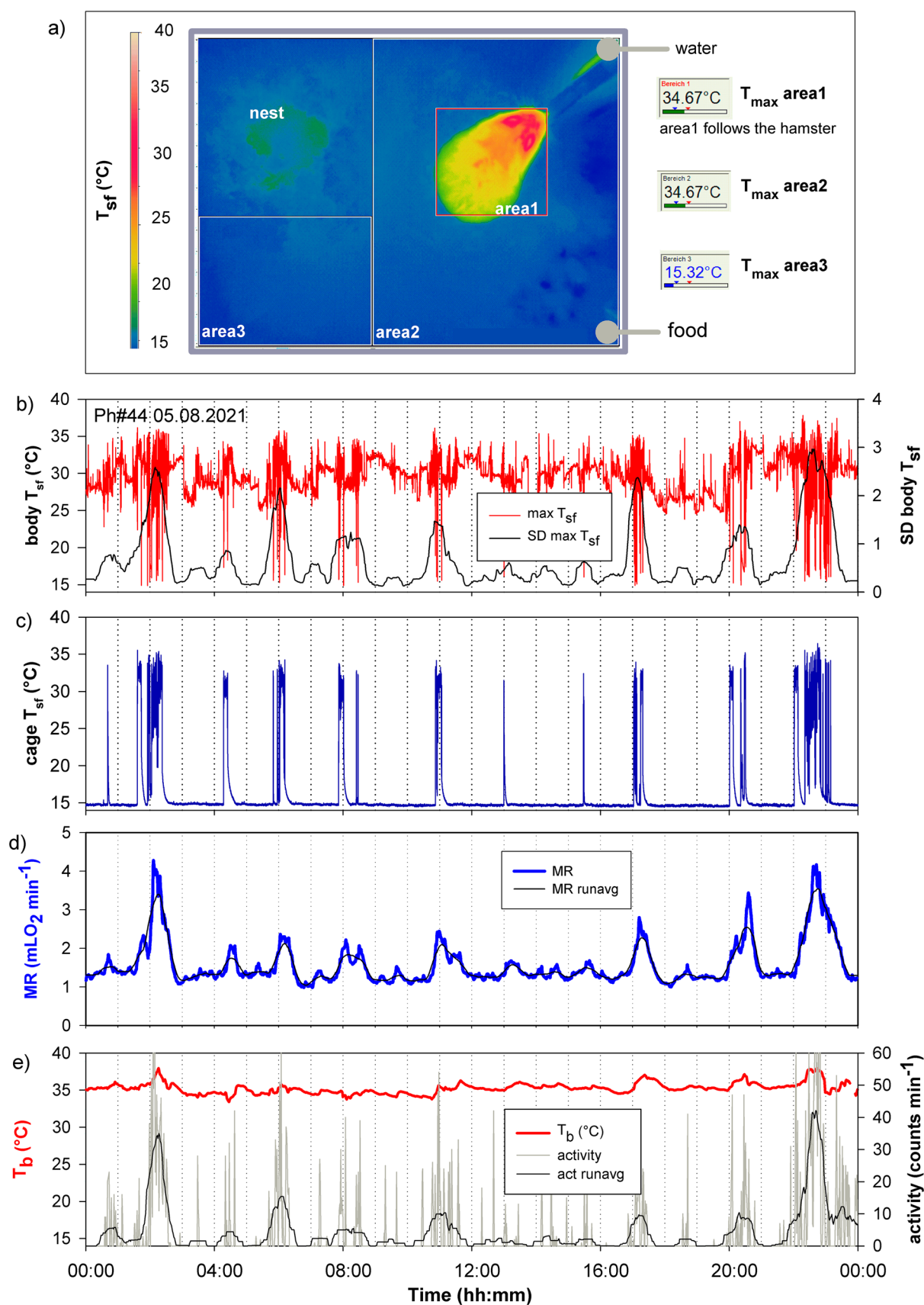


Fig. 10 Activity behaviour of a hamster (Ph#44, male, Mb 28.6 g) recorded with an infrared thermovision camera (setup see Fig. 10a). Pictures were scanned and stored in one second intervals. Three areas of interest were defined. Area1 was programmed to follow the hamster, area2 and area3 were fixed and covered the cage bottom outside the nest. Maximum T_{sf} was read every second from the hamster (Fig. 10b, red line) and the cage bottom. T_{sf} of eyes and face of the hamster are close to T_b recorded with implanted transmitters (Fig. 10b). Most of the time hamster T_{sf} was below T_b (Fig. 10e) because only the fur surface was in sight of the thermovision camera. SD for body T_{sf} was calculated as an index for activity (Fig. 10b black line). Cage T_{sf} showed stable values at $\sim 15^\circ\text{C}$, interrupted by rises above 30°C which happened when hamster was active outside the nest (Fig. 10c). MR of the hamster (Fig. 10d). The blue line are readings per min and the black line is average MR (30 min running average) derived from the readings per min. Figure 10 e: core T_b of the hamster (red line) and locomotor activity both measured with transmitter telemetry. Activity is presented with two lines. Activity counts per min (grey line) and 30 min running average of activity counts per min (black line)

study further shows that wavelet analysis including a power spectrum provides a more detailed insight into the dynamics of URs than Fourier analysis, Lomb-Scargle periodograms or other methods of time series analysis.

URs measured on the cellular level or in biochemical pathways can be shorter than systemic URs. Protein biosynthesis in dense cultures of rat hepatocytes cycle with periods ranging between 30 min and 1 h (Brodsky 2014). Similar URs were found in the dry weight of acinar cells of the parotid gland in rats or in UV-absorption of crawfish mechanoreceptor neurons (Brodsky 2014). Similar short period URs were observed in blood levels of insulin and catecholamines (Simon and Brandenberger 2002; Schöfl et al 1997).

The day-by-day variation of URs occurred in all three URs to a similar extent (Fig. 3). The variability of their correlation suggests that there are at least three

autonomous UR oscillators which are mutually synchronized or switched on and off by a network of interactions, instead of being a fraction or harmonic of one single UR (Fig. 4). The existence of an ultradian master clock, similar to the SCN for circadian rhythms, is unlikely, at least at the present state of knowledge. However, there is evidence for the coordination of URs on the cellular and tissue level (Isomura and Kageyama 2014; Yang et al. 2022) as well an interaction of several brain areas and endocrine networks (Grant et al. 2018; Goh et al. 2019) which could be held responsible for URs with different periods. These activities may be superimposed by the dopamine ultradian oscillator (DUO) which controls arousal, sleep and activity in a period range of ~ 4 h (Blum et al. 2014; Prendergast and Zucker 2016). This periodicity corresponds with the UR_{large} in our present study.

Neural control of ultradian rhythms

Our present findings indicate that several URs are oscillating in an animal at the same time, in cells as well as on the systemic level. This raises the question if they are running in parallel, independent from each other, or if they are linked by endocrine or neural control. The 24hourly records of MR, T_b and activity records in Fig. 1 show high amplitude URs during the nocturnal activity phase and low amplitudes during the diurnal resting phase, indicating an interaction between the circadian and the ultradian system.

Ablation of the circadian pacemaker in the SCN eliminates CRs but URs of activity behaviour and feeding persisted in 8 out of 11 SCN-ablated voles (Gerkema et al. 1990b, Gerkema and van der Leest 1991). Similar observations were made in golden hamsters and rats (Refinetti et al 1994; Stephan and Zucker 1972). In contrast SCN ablation in LEW/Ztm rats abolished circadian as well as ultradian

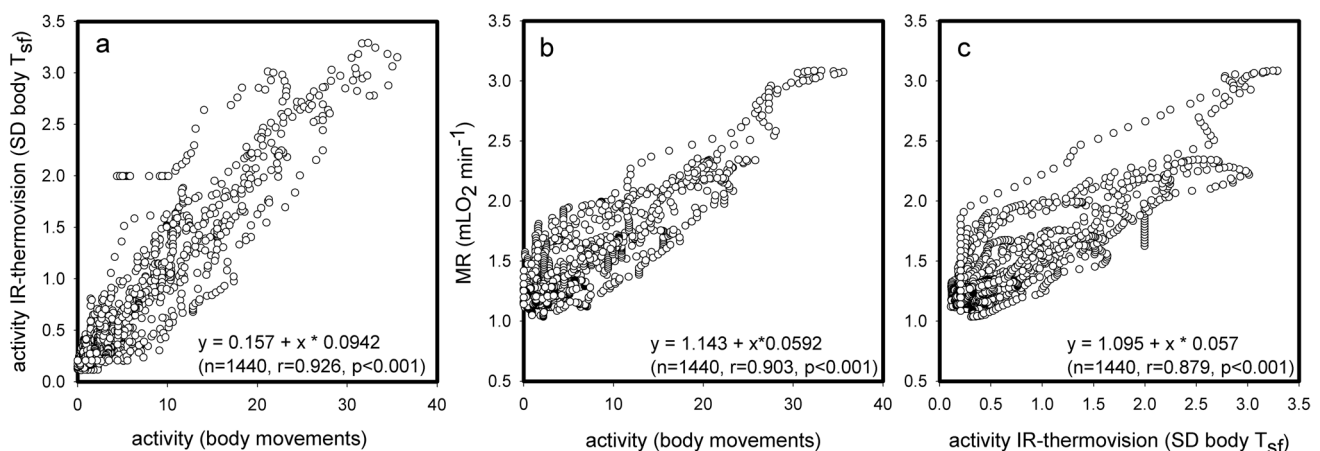


Fig. 11 Comparison of locomotor activity measured by transmitter and infrared thermovision (Fig. 10a). Relation between MR and activity either measured with implanted transmitter (Fig. 10e) or infrared thermovision (Fig. 10b)

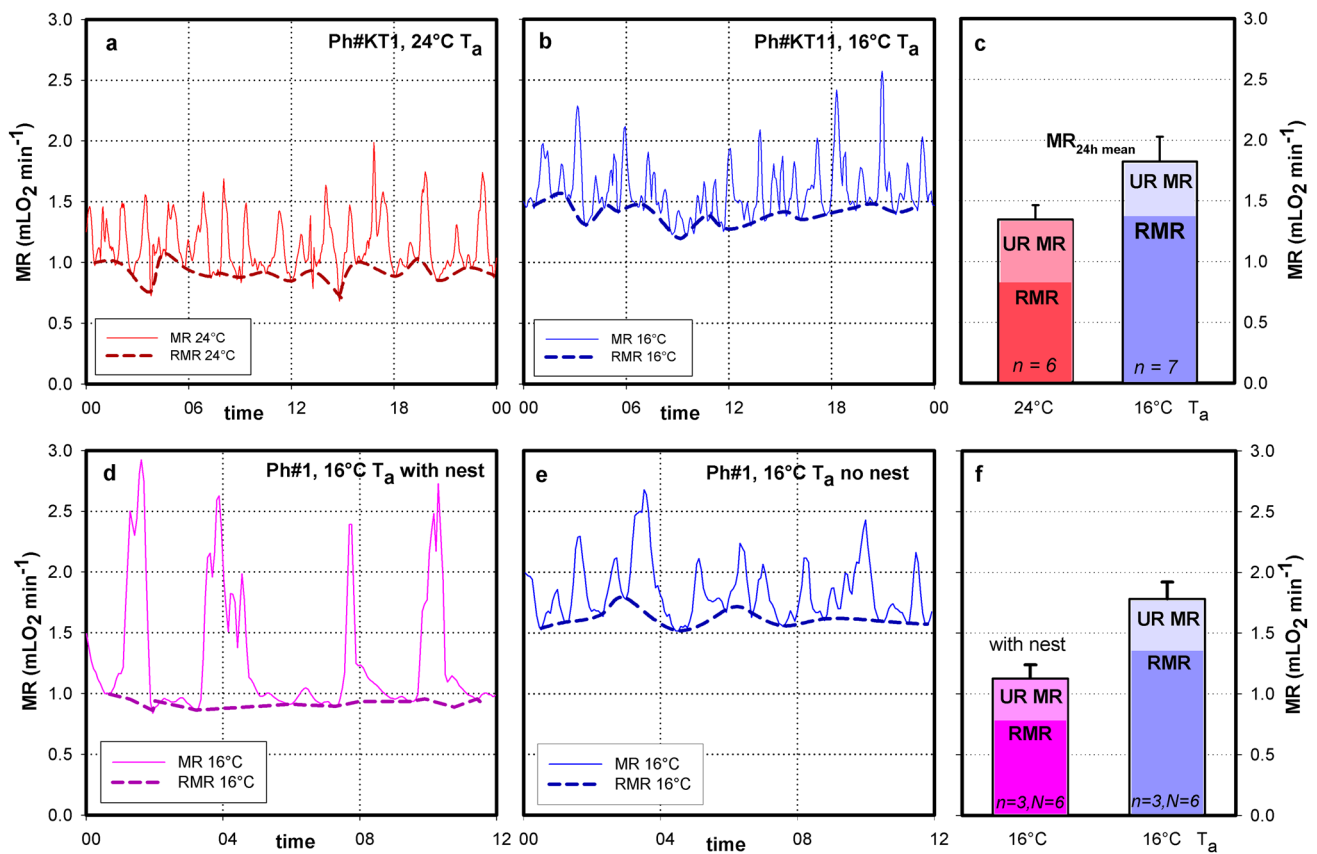


Fig. 12 Effect of cold and nest building on hamster energy requirements. Panels a, b show 24 h records of MR of two hamsters at 24 °C (pink) or 16 °C T_a (blue), including RMR calculated from minimum MR (6 min averages). Graph c mean values for MR, RMR and UR

MR. See also Table 1. Graph c, d 12 h records of one hamster at 16 °C T_a with nest (pink) and 16 °C without nest. Graph f mean values from 3 hamsters and 6 recordings. See also Table 2

components of activity rhythms (Wollnik and Turek 1989). The discrepancy may be due to the extent of surgical ablation in the hypothalamus. Detailed histological analyses of ablation studies in voles (Gerkema et al. 1990b) showed that hypothalamic regions outside the SCN were generating URs

which exerted ultradian effects on the SCN. In free running voles ultradian activity bursts were phase locked with CRs (Gerkema et al. 1993). This was confirmed by the analysis of calcium URs in hypothalamic mouse brain slices indicating

Table 3 Energy expenditure and metabolic URs of Djungarian hamsters at 24 °C T_a (thermoneutral) and 7 hamsters at 16 °C T_a (moderate cold). T_a was measured inside the cages. The differences between the two groups were compared by t-tests

Ambient temperature (T_a)	24 °C (n=6)	16 °C (n=7)	
DEE [kJ d^{-1}]	34.8 ± 13.9	58.1 ± 6.5	$p=0.001$
HP _{24h} mean [mW]	403.2 ± 140.5	671.4 ± 75.7	$p=0.001$
MR _{24h} mean [$\text{mLO}_2 \text{ min}^{-1}$]	1.345 ± 0.118	1.822 ± 0.204	$p<0.001$
RMR _{24h} mean [$\text{mLO}_2 \text{ min}^{-1}$]	0.845 ± 0.126	1.423 ± 0.249	$p=0.001$
Ultradian MR _{24h} mean [$\text{mLO}_2 \text{ min}^{-1}$]	0.511 ± 0.120	0.409 ± 0.129	$n.s$
Ultradian MR as % of MR _{24h} mean	37.9%	22.4%	
RER	0.928 ± 0.029	0.893 ± 0.034	$n.s$
Mean peak MR [$\text{mLO}_2 \text{ min}^{-1}$]	1.625 ± 0.149	2.129 ± 0.226	$p<0.001$
Number of peaks [n]	33.5 ± 1.4	31.3 ± 6.7	$n.s$
Period UR _{small} [h]	0.803 ± 0.128	0.837 ± 0.208	$n.s$
Period UR _{medium} [h]	1.345 ± 0.217	1.507 ± 0.515	$n.s$
Period UR _{large} [h]	2.312 ± 0.458	2.673 ± 0.971	$n.s$

Table 4 Metabolic rate and metabolic URs of Djungarian hamsters at 16 °C either without or with a nest. Results from 12 h records in three hamsters, each hamster was recorded twice (n = 3, N = 6). Mean body mass was 28.4 g. Means $\pm$ SD

	T _a 16 °C (n = 3, N = 6) no nest	T _a 16 °C with nest
T _b 12 h mean	35.9 $\pm$ 0.3	35.6 $\pm$ 0.1
MR _{12h} mean [mLO ₂ min ⁻¹]	1.782 $\pm$ 0.204*	1.092 $\pm$ 0.140*
RMR _{12h} mean [mLO ₂ min ⁻¹]	1.415 $\pm$ 0.207*	0.829 $\pm$ 0.091*
Ultradian MR _{12h} mean [mLO ₂ min ⁻¹]	0.297 $\pm$ 0.080	0.263 $\pm$ 0.068
Ultradian MR as % of MR _{12h} mean	16.0%	24.1%
RER _{12h} mean	0.947 $\pm$ 0.018	0.946 $\pm$ 0.018
Average peak MR [mLO ₂ min ⁻¹]	2.124 $\pm$ 0.301*	1.624 $\pm$ 0.072*
Number of peaks per 12 h > 2 mLO ₂ min ⁻¹	10.25 $\pm$ 3.63*	4.00 $\pm$ 1.77*
Period UR _{small} [h]	0.772 $\pm$ 0.245	0.892 $\pm$ 0.099
Period UR _{medium} [h]	1.312 $\pm$ 0.433	1.508 $\pm$ 0.054
Period UR _{large} period [h]	2.418 $\pm$ 0.694	2.439 $\pm$ 0.006

*Values differ significantly in hamsters kept with and without nest $p < 0.05$

that URs originated in the SPZ-PVN region which stimulates ultradian activity of the SCN (Wu et al. 2018).

The striatum, a part of the basal ganglia, is a further important player in the expression of URs. Dopamine levels in the striatum of mice, obtained with microdialysis, showed ultradian fluctuations with a period of about 2.5 to 3 h in parallel with URs of locomotor activity. High levels of dopamine correlate with high levels of activity. Knock-out of the dopamine transporter lengthened the ultradian activity period from 4 to 14 h (Blum et al. 2014). Methamphetamine, which strongly affects brain dopamine levels, prolongs the period of ultradian activity periods in a dose dependent manner, concluding that dopaminergic signalling in the striatum plays a leading role in the expression of URs of rest and activity behaviour (Blum et al. 2014; Bourguignon and Storch 2017), suggesting the existence of a Dopaminergic Ultradian Oscillator (DUO, Blume et al. 2014). At present it is unknown how the striatal dopaminergic signalling and hypothalamic nuclei are interacting with each other to achieve control of systemic URs, as exemplified by the UR_{large} in our present study.

Catecholamines play an important role in the expression of brain URs. The release rates of catecholamines and histamine, as well as GABA, glutamate and NO levels fluctuated in an ultradian manner in several brain areas (Philippu et al. 1979; Philippu 2016). These coincided with rhythmic fluctuations of the EEG. Central application of catecholamine and histamine receptor agonists and antagonists modulated the ultradian EEG pattern (Grass et al. 1995; Philippu 2019). Electrocoagulation of the rostral arcuate nucleus abolished ultradian activities suggesting that this brain area plays a central role for brain URs (Grass et al. 1996). This underlines the compendious role of ultradian rhythms for general information processing and the state of vigilance of an animal.

Endocrine control of ultradian rhythms

In our present study we identified two further URs with shorter periods, UR_{small} and UR_{medium}, which may be linked to endocrine effects on peripheral metabolism. Endocrines which could affect MR in peripheral tissues show a wide range of URs (for review see Grant et al. 2018). Insulin is considered as the major anabolic hormone of the body, responsible for the absorption of glucose from blood into the liver, fat and skeletal muscle and its conversion and storage as glycogen and lipid. Despite its acute response to food intake, it has basal URs with periods of 10 min and 50 min in humans (Simon and Brandenberger 2002) and 5–17 min plus 50–150 min in rats (Chou et al. 1994) or 26 min in obese rats (Otukonyong et al. 2005). The short period length of ~ 10 min is probably generated by the pancreatic islands themselves (Chou et al. 1994). Similar periodicities were also reported for glucagon, ghrelin and leptin indicating that energy balance is under control of URs with short periods.

The metabolic effects of catecholamines include mobilization of glucose from glycogen stores. Catecholamine levels in the blood of human volunteers changed with an UR of 50–100 min (Schöfl et al. 1997). Similar short periods were reported for the release of noradrenaline (54 min), dopamine (37 min) and adrenaline (36 min) from the Locus coeruleus of cats (Singewald et al. 1994). In the hypothalamus of rats, pulsatile release rates of noradrenaline (60–120 min, mean 92 $\pm$ 4 min), adrenaline (99 min) and dopamine (92 min) could be detected, whereby the releases of adrenaline and noradrenaline coincided to a high extent (Dietl et al. 1993). Elevated levels of peripheral noradrenaline facilitated lipolysis and induced nonshivering thermogenesis in brown adipose tissue causing an immediate rise of MR and T_b (Oot-suoka et al. 2009, Blessing et al. 2012).

Glucocorticoids mediate, on a short time scale, glucose availability and lipolysis, and on a long time scale, gene transcription and immune responses. The hypothalamo-pituitary-adrenal axis (HPA) is characterized by a pulsatile release of CRH, ACTH and adrenal release of glucocorticoids (CORT) at about hourly intervals (Jasper and Engeland 1991; Mershon et al. 1992; Spiga et al. 2011; Flynn et al. 2018).

Thyroid hormones affect basal MR and protein biosynthesis. In dairy cows and humans thyroid hormone levels showed URs of about 1.5 h (Bitman et al. 1994). A similar periodicity of pulsatile release of TSH was reported in mares and humans (Buff et al. 2007; Roelfsema et al. 2017) whereas the level of TRH in rats showed an ultradian periodicity of more than 4 h (5.8 pulses in 24 h) (Okauchi et al. 1996) which would be twice as long as the UR period of TSH and the thyroid hormones T4/T3. In a further study on rats TRH and TSH were measured simultaneously (Mizobuchi et al. 1996) confirming this discrepancy, which gave rise to the suggestion that TSH oscillates with double the frequency of TRH (Grant et al. 2018). The evidence for this however is quite small and is based on rat brain microdialysis with hourly sampling intervals, which limits the resolution for measuring URs.

Insulin, catecholamines, CORT, and T4/T3 activate or control metabolism in peripheral tissues on a short time scale between 0.5 and 2 h. Hypothetically they could be involved in the expression of URs. The stable constellation of the three URs even in energetically challenged hamsters, suggests that the endocrine system itself is part of the expression of URs rather than the cause of URs. This underlines the ubiquitous role of URs in control of metabolic processes in cells, tissues and the entire organism. MR is usually considered as a proximate requirement to cover the energy requirements of an animal for thermoregulation and activity. The present findings show that this is only part of the truth. RMR is adjusted to cover the needs of thermoregulation. On top of RMR metabolic bursts are added to cover the needs of activity, behaviour and metabolic processes requiring energy. The latter are under control of endogenous URs which have major effects on total energy balance of an animal to an extent which needs yet to be explored.

Conclusions

We identified three different ultradian rhythms, instead of only one previously known from Djungarian hamsters and other mammals. They are characterized by different period length and amplitude, URsmall (period ~ 1 h), URmedium (1.5–2.2 h) and URlarge (~ 2.0–5.0 h). Wavelet analysis showed that these URs are present in the time course of metabolic rate, body temperature, and locomotor activity.

The three ultradian rhythms are running in parallel and may overlap each other. Their period varies between individuals as well as in each individual from day to day. In long term records the periods of all three URs were observed changing in parallel. This suggests that URs are controlled by separate ultradian oscillators interacting with each other. They are not part of the circadian system, but the lower amplitude of ultradian rhythms during the diurnal resting phase indicates an interaction between circadian rhythms and the expression of ultradian rhythms. Ultradian rhythms of metabolic rate require up to 38% of the daily energy budget. This underlines the significance of rheostasis for control of metabolism, thermoregulation and locomotor activity.

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Declarations

Conflict of interest GH has developed the CaloBox which is produced and sold by PhenoSys GmbH, Berlin. GH has a competing interest as EIC of Journal of Comparative Physiology B.

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