ジャンガリアンハムスター（*Phodopus sungorus*）の体温リズムと歩行活動リズムにおける日内休眠と関連した概日およびウルトラジアン成分の分離発現

Differential Expression of Circadian and Ultradian Components in the Body Temperature and Locomotor Activity Rhythms in Relation to Daily Torpor of the Djungarian Hamster (*Phodopus sungorus*)

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The Djungarian hamster shows daily torpor that occurs in winter with decreased body temperature (about 10-20℃) during the daytime. We tried to elucidate the characteristics of circadian and ultradian body temperature and locomotor activity rhythms in torpid and non-torpid states. Adult male hamsters were kept in long photoperiod and high temperature (LP-HT) before the experiment and, thereafter, animals were transferred to short photoperiod and low temperature (SP-LT) and were kept in this condition for about six months. Body weight and food intake were monitored throughout the experiment. The rhythms of locomotor activity and body temperature were recorded every three or five minutes by using a telemetry system. There were two types of animals in the response to SP-LT, i. e., torpid animals that showed daily torpor with reduction of body weight and non-torpid animals that did not show daily torpor without reduction of body weight. Non-torpid animals always exhibited circadian rhythms in both body temperature and locomotor activity rhythms throughout the experimental period. Torpid animals, on the other hand, expressed a circadian component in body temperature rhythms and both circadian and ultradian components in locomotor activity rhythms during torpid days. Torpid animals during non-torpid days, however, showed both circadian and ultradian components in body temperature rhythms and only ultradian component in locomotor activity rhythms. We discussed the functional significance of differential expression of circadian and ultradian rhythms.

Key Words: :daily torpor, locomotor activity, body temperature, circadian rhythm, ultradian rhythm,

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Introduction

Changes in photoperiods are important and reliable to predict seasonal variation in behavior and physiology such as gonadal recrudescence, fur color change, metabolism, hibernation, etc. (Masuda and Oishi, 1988; 1989; Tsutsui et al., 1988; 1989; Goldman, 2001).

Hibernation is a phenomenon in that homeothermic animals reduce their body temperature close to 0 ℃ to cope with severe winter. Hibernating animals set up continuous hibernation (bout) and arousal (periodic arousal) alternately during hibernation. Daily torpor, on the other hand, is a phenomenon in that body temperature shows bout and periodic arousal daily with reduction of body temperature more than 10 ℃ to become torpid state (Tucker, 1962; Geiser and Ruf, 1995).

Daily torpor is probably adaptation to winter for small mammals to reduce their daily energy expenditure. In some species, torpor can be facultatively induced by food restriction and moderate cold exposure (Tannenbaum and Pivorun, 1987). However, in strictly photoperiodic species, torpor occurs only after several weeks of exposure to a short-day photoperiod and cannot be provoked by cold exposure and/or moderate food restriction. Previous studies showed that the Djungarian hamster maintained in short photoperiods induced torpor regularly during their circadian resting period, even when they were kept at thermoneutrality and fed *ad lib* (Heldmaier and Steinlechner, 1981). Thus, they display torpor spontaneously without any acute shortage of energy supplies and spontaneously reduce their daily energy expenses (Ruf and Heldmaier, 1992a, b; Heldmaier, 1989). A circanual cycle of reproduction is exhibited in a small percentage of Siberian hamsters chronically exposed to short days (Anchordoquy and Lynch, 2000).This spontaneous torpor is generated by the suprachiasmatic nucleus (SCN), because ablation of the SCN eliminates it (Ruby et al., 1989). However, there are some individuals which do not enter torpor in the same condition. So, they can be separated into photo-responsive and photo-non-responsive phenotypes (Puchalski and Lynch, 1986). These phenotypes have been studied from points of genetics and photoperiodic history (Goldman et al., 2000). Variations in photoperiodic response are seen not only between species but also between breeding populations within a species and between individuals within single breeding populations (Goldman, 2001). But the differences in the pattern of rhythms have not been compared between photo-responsive and photo-non-responsive phenotypes nor between torpid and non-torpid states. In the present study, we measured locomotor activity and body temperature rhythms and compared the differences between torpid and non-torpid animals.

Materials and Methods

Animals and experimental conditions

At one month of age, 30 Djungarian hamsters, *Phodopus sungorus*, were obtained (Midorien, Japan) and housed singly in plastic cages (25×18×13 cm) containing wood shavings with steel mesh lids for about two months. Food and water were given *ad lib*. They were fed rodent pellets (CE-2, CLEA, Japan). Animals were maintained in long-day photoperiod and high temperature (LP: 16h light and 8h darkness, light on: 8:00-24:00, HT: 24±2 ℃) for one month, and then they were transferred to short-day photoperiod and low temperature (SP: 8h light and 16h darkness, light on: 10:00-18:00, LT: 10±2 ℃) and maintained in this condition for 24 weeks.

Measurement of locomotor activity and body temperature

Locomotor activity and body temperature were measured by a telemetry system (MiniMitter, model XM-FH, Oregon, USA). Telemeters were implanted into the abdominal cavity of animals (n=16) under diluted Nembtal anesthesia (Nembtal: Propylene glycol: Ethanol: DDW= 3: 4: 2: 7). After the surgery, they were allowed to recover for 4 days. Locomotor activity and body temperature were recorded every three or five minutes using a personal computer (Aptiva, IBM) and software (DataquestⅢ, Data Sciences International or Vital View, MiniMitter). The battery of the telemetry was exchanged every two months. Circadian and ultradian rhythms were analyzed by the fast Fourier transformation (FFT) software and X^2-periodogram. Body weight and food intake were measured once a week for about six months. The data were analyzed by ANOVA with PLSD.

Terms were defined as follows. Non-torpid animals mean the animals that did not show daily torpor throughout the experimental period. Torpid animals mean the animals that showed daily torpor. Torpid days mean the days when the torpid animals showed daily torpor. Non-torpid days mean the days when the torpid animals did not show daily torpor.

Results

Six animals exhibited torpor (torpid animals) and others did not (non-torpid animals). We implanted a telemeter into 16 non-torpid animals, but the animals with continuously recorded data were only 6. We implanted telemetry for torpid animals when they showed torpor.

Body weight and Food intake

Fig.1 shows the changes of body weight and food intake during the experiment. Non-torpid animals maintained the initial body weight throughout the experiment. In contrast, in torpid animals, body weight began to decrease after the animals were transferred from LP-HT to SP-LT. It became significantly low after the 8th week as compared with that at the beginning of experiment (p<0.05) and it began to increase after the 18th week (Fig.1 A). Both torpid and non-torpid animals increased their food intake just after they were transferred from LP-HT to SP-LT and the amount of food intake leveled off at about 4 weeks after the transfer. The non-torpid animals maintained the large amount of food intake thereafter, whereas food intake of torpid animals was decreased from the 13th to the 18th week because of daily torpor (Fig.1 B).
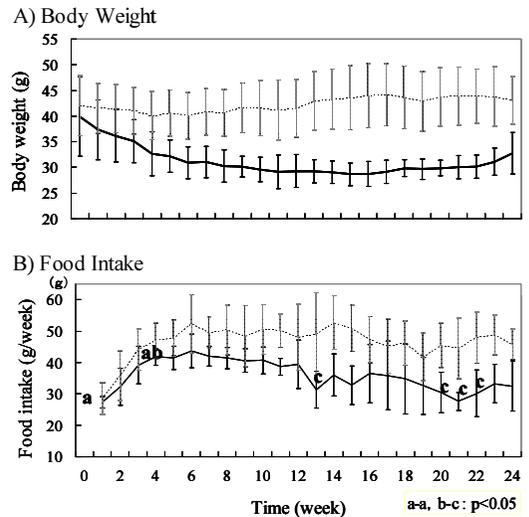

Fig.1. Body weight (A) and food intake (B) of Djungarian hamsters after they were transferred from long photoperiod-high temperature to short photoperiod-low temperature. Solid line: torpid animals. Dotted line: non-torpid animals. Vertical bars indicate standard deviation.

Locomotor activity and Body temperature rhythms

In long day photoperiod and high temperature (LP-HT), hamsters showed circadian rhythms in

A) Body temperature and locomotor activity rhythms

B) Power spectra of body temperature and locomotor activity rhythms
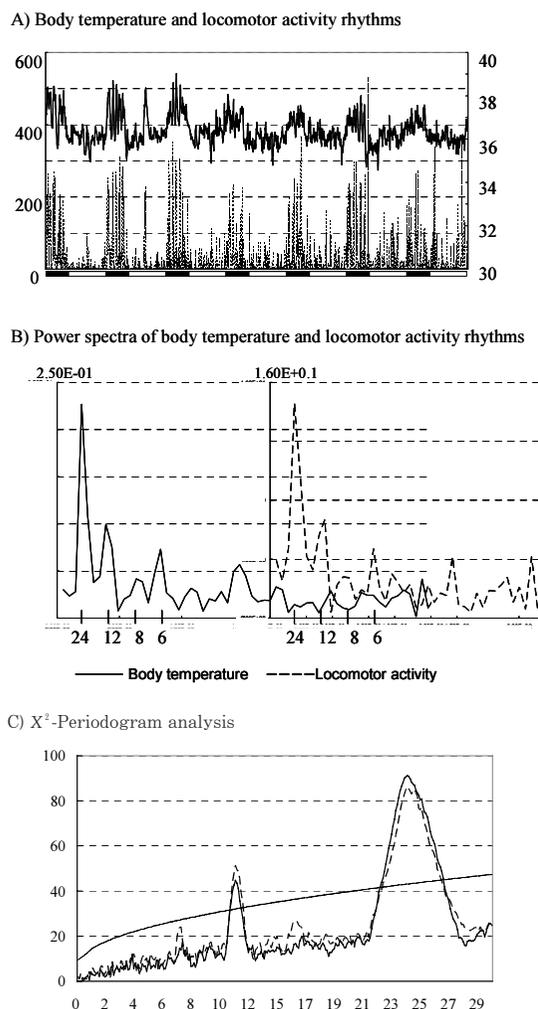
C) X^2-Periodogram analysis

Fig. 2.   Results obtained in hamsters under long photoperiod-high temperature. (A) The solid line shows body temperature (℃) and dotted bars show the amount of locomotor activity per 5min. Horizontal white bars and black bars beneath the graph indicate day and night, respectively. (B) Power spectra analysis of body temperature and locomotor activity. (C) X^2-periodogram analysis.

both locomotor activity and body temperature (Fig. 2A). Power spectra analysis (FFT) revealed that the animals had only circadian component in both locomotor and body temperature rhythms (Fig. 2B). Power spectra analysis were followed by X^2-periodogram analysis (Fig.2C). There were two types (torpid and non-torpid animals) in the response of body temperature rhythms to short-day photoperiod and low temperature (SP-LT). Six animals exhibited daily torpor (torpid), while 24 animals did not (non-torpid).

In torpid animals, circadian components in both body temperature and locomotor activity rhythms were lost, and the rhythms with a 12 hour period and ultradian components (< 6 hours) appeared at 3 weeks before the occurrence of daily torpor (about 10 weeks after they were transferred to SP-LT from LP-HT) (Fig. 3A and B). However, when they started to exhibit daily torpor with decreased body weight, circadian components reappeared. During this period, there were two types of days with exhibition of daily torpor (torpid days) and without exhibition of daily torpor (non-torpid days) (Fig.3). In torpid days, body temperature started to decrease at the end of dark phase in coincidence with the decrease of locomotor activity, and increased rapidly at the end of light phase in coincidence with the increase of locomotor activity. The ultradian component appeared only in locomotor activity rhythms during torpid days, while the circadian component was observed in both body temperature and locomotor activity rhythms (Table 1, Fig. 3). In non-torpid days, on the other hand, the ultradian components appeared in both body temperature and locomotor activity rhythms, while the circadian component disappeared in locomotor activity rhythms except one animal.   Thus, appearance of ultradian and
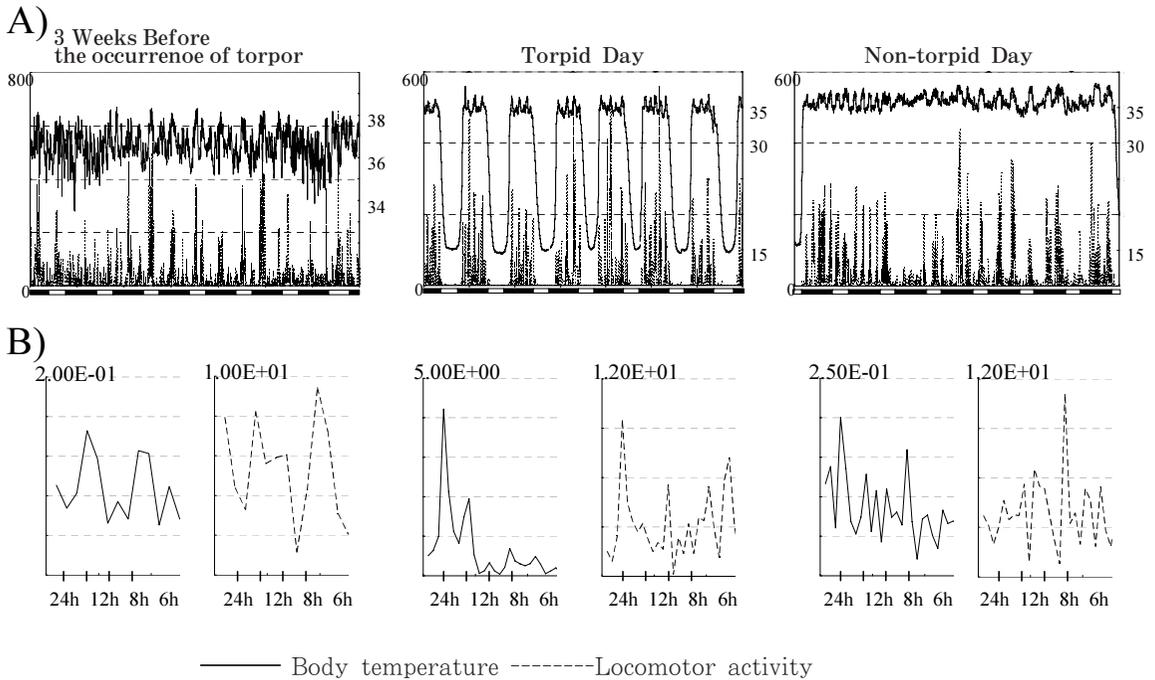
Fig. 3.　(A) Body temperature (℃) and locomotor activity in torpid animals.　The rhythm in an animal 3 weeks before the occurrence of daily torpor (left panel), torpid days (middle panel) and non-torpid days (right panel).　(B) Power spectrum analysis of body temperature and locomotor activity.

Table 1. The number of hamsters that showed a circadian and/or ultradian rhythm under short day photoperiod and low temperature in torpid and non-torpid animals.

			Circadian rhythm (24h)	Ultradian rhythm (<6h)
Non-torpid animals n=6	Locomotor activity		6	0
	Body temperature		6	0
Torpid animals n=6	Torpid day	Locomotor activity	6	6
		Body temperature	6	0
	Non-torpid day	Locomotor activity	1	6
		Body temperature	6	6

circadian rhythms differed depending on torpid and non-torpid days and also depending on locomotor activity and body temperature (Table 1). The results of power spectra analysis were followed by X^2-periodogram analysis.

In non-torpid animals, we compared the locomotor activity and body temperature rhythms at 3, 8 and 12 weeks after the transfer to SP-LT (Fig. 4A). The peaks of locomotor activity and body temperature were in the middle of dark phase at 5 weeks after the transfer to SP-LT and they gradually shifted toward the end of dark phase (Fig. 5). Power spectra analysis detected only circadian components in both locomotor activity and body temperature rhythms throughout the experimental period (Fig. 4B, Table 1).

After 5weeks in LT-SP (the average of 5days)
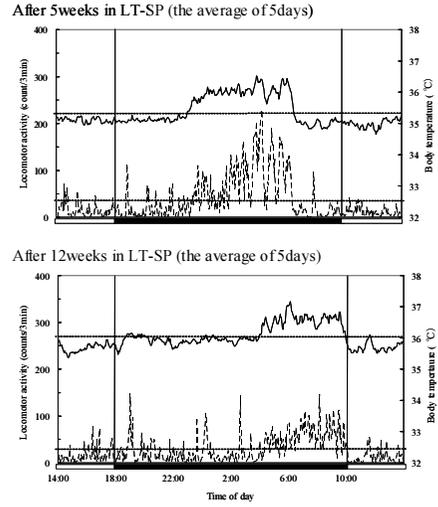
After 12weeks in LT-SP (the average of 5days)

Fig. 5　The phase of body temperature and locomotor activity rhythms after 3 weeks in short photoperiod-low temperature delayed from the middle of night towards the end of night after 12 weeks in short photoperiod-low temperature.
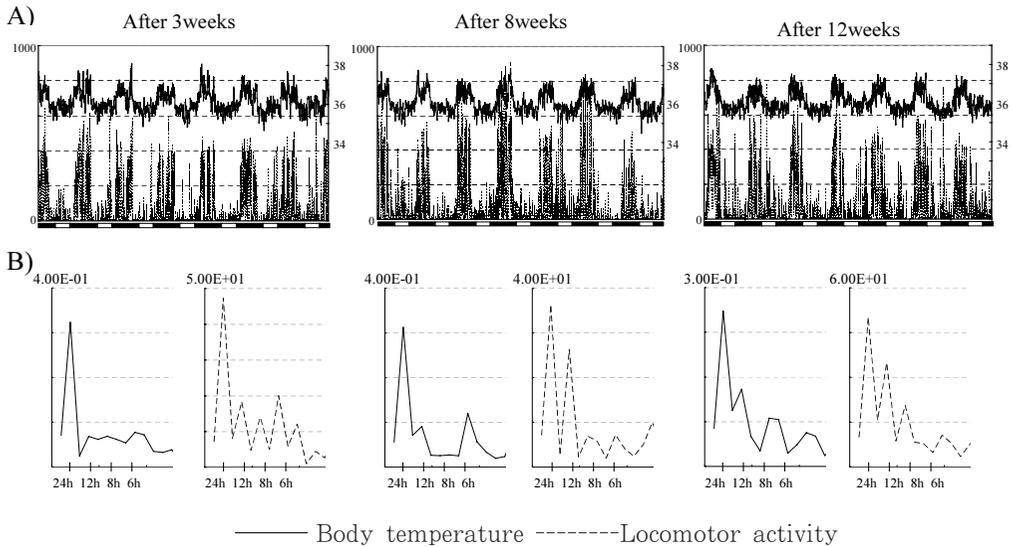
A)

After 3weeks　　　　After 8weeks　　　　After 12weeks

B)

—— Body temperature ------- Locomotor activity

Fig. 4.　(A) Body temperature (℃) and locomotor activity in non-torpid animals.　The rhythm in an animal 3 weeks, 8 weeks and 12 weeks after the transfer to short photoperiod-low temperature condition.　(B) Power spectrum analysis of body temperature and locomotor activity.

Discussion

The Djungarian hamster, *Phodopus sungorus*, exhibits body weight loss, gonadal regression, and pelage color changes from brown to white and daily torpor in short-day photoperiod (Heldmaier and Steinlechner, 1981; Hoffmann, 1973). In the present study, hamsters consumed a large amount of food for keeping their body temperature in cold ambient temperature. In torpid animals, food intake was decreased from the 13th to the 18th week because of daily torpor. This result supports the idea that daily torpor is to reduce their daily energy expenditure (Heldmaier, 1989).

The response to short-day photoperiod has been shown to depend on individuals, and, thus, the hamsters can be separated into photo-responsive and photo-non-responsive phenotypes as reported by Puchalski and Lynch (1986). Only six out of 30 animals showed body weight loss and expression of daily torpor in the present experiment, and, therefore, most hamsters we used were the photo-non-responsive type. In our previous experiments (Jefimow et al., 2004), however, most hamsters exhibited daily torpor under short photoperiod. The reason for this difference is probably the difference in prior photoperiod history, because incidence of nonresponsiveness was significantly higher in 18L6D hamsters than in 15L9D hamsters (Prendergast and Freeman, 1999). Goldman et al. (2000) also reported that hamsters that have been reared in an exceptionally long day length (18L) do not usually exhibit the winter phenotype after transfer to short days, whereas animals reared under moderately long days (16 L) are more variable in responsiveness to subsequent short day exposure and hamsters reared exclusively in an "intermediate" day length (14 L) are almost uniformly responsive to short photoperiod.

A recent study in four diurnal and four nocturnal small rodent species showed that the daily rhythms of locomotor activity and body temperature were synchronized and both rhythms have highly significant correlation (Refinetti, 1999). Our results also showed that the daily rhythms of body temperature and locomotor activity are very closely synchronized in LP-HT and in non-torpid animals in SP-LT. However, torpid animals in SP-LT showed differencial expression of circadian and ultradian components between locomotor and body temperature rhythms. Both circadian and ultradian rhythms were exhibited in the locomotor activity during torpid days, while only circadian rhythm was distinct in the body temperature rhythm. On the other hand, opposite results were obtained during non-torpid days; both circadian and ultradian rhythms were exhibited in the body temperature rhythm and only ultradian rhythm was distinct in the locomotor activity rhythm.

Large magnitude of phase advance induced arrhythmicity or freerunning rhythms in body temperature and locomotor activity rhythms in Siberian hamsters (Ruby et al., 1998). Steinlechner et al. (2002) reported that the locomotor activity, body temperature and melatonin rhythms in Djungarian hamsters were lost and became arrhythmic under light and dark cycles with short light pulses. They assume that the pacemaker of Djungarian hamsters can be driven to a state of zero phase difference between the two oscillators due to loose coupling with zero amplitude of their outputs. They did not analyze the ultradian components in their experiments, but the arrhythmicity they observed might have some relation to the torpid animals which lost the circadian

component and expressed only ultradian components in the locomotor activity rhythms in non-torpid days. We also found that the Japanese grass vole (*Microtus montebelli*) shows ultradian rhythms when they switch their activity from nocturnal in summer to diurnal in winter seasonally (Matsuoka et al., unpublished).

Studies on circadian and ultradian rhythms have been done in many species of small rodents. In rats, restricted feeding induced disappearance of circadian rhythms and appearance of ultradian rhythms (Widman and Timberlake, 1995). The Djungarian hamster increased their activity and continuously active with loss of the circadian rhythm under both long and short day photoperiods when food was restricted (Masuda and Oishi, 1995). Similar results on the effects of restricted feeding were obtained in voles (Daan and Slopaema, 1978) and Djungarian hamsters (Masuda and Oishi, 1995). Paul et al. (2004) recently suggested that the circadian pacemaker in the suprachiasmatic nuclei (SCN) controls the time of torpor onset indirectly by affecting timing of food intake, rather than by, or in addition to, direct neural and humoral outputs to relevant target tissues. Expression of ultradian rhythms and disappearance of circadian rhythms may have relation to changes in modes of food consumption under torpidity which is similar to food restriction. Since various stresses induce ultradian rhythms in rats, there is also a possibility that body temperature reduction due to torpor may have stressful effects to induce ultradian rhythms.

Since Honma et al. (1988) reported that lesion of SCN in rats abolished both circadian and ultradian rhythms in locomotor activity, body temperature and plasma corticosterone levels, SCN may be the ultradian oscillator as well as the circadian oscillator. Gerkema et al. (1990), however, revealed that circadian rhythms are abolished by SCN lesion, but that lesion of both or one of the retrochiasmatic area and adjacent arcuate nucleus did not abolish ultradian rhythms in common voles. Therefore, it is necessary to locate the oscillator of the ultradian rhythms in Djungarian hamsters.

In the present study, differential expression of circadian and ultradian rhythms was observed in locomotor activity and body temperature, but the function of such differential expression remains to be investigated in future experiments.

References

Anchordoquy HC and Lynch GR (2000) Evidence of an annual rhythm in a small proportion of Siberian hamsters exposed to chronic short days. J. Biol. Rhythms 15:122-125.

Daan S and Slopaema S (1978) Short term rhythms in foraging behavior of the common vole, *Microtus arvalis*. J. Comp. Physiol. 127:R149-155.

Geiser F and Ruf T (1995) Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. Physiol. Zool. 68:935-966.

Gerkema MP, Groos GA, and Daan S (1990) Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. J. Biol. Rhythms 5:81-95.

Goldman BD (2001) Mammalian photoperiodic system: Formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J. Biol. Rhythms 16:283-301.

Goldman SL, Dhandapani K and Goldman BD (2000) Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Photopus sungorus*). J. Biol. Rhythms

15: 417-428.

Heldmaier G (1989) Photoperiod and thermoregulation in vertebrates: body temperature rhythms and thermogenic acclimation. J. Biol. Rhythms 4:251-265.

Heldmaier G and Steinlechner S (1981) Seasonal control of energy requirements for thermoregulation in the Djungarian hamster (*Phodopus sungorus*), living in natural photoperiod. J. Comp. Physiol. 142:429-437.

Hoffmann K (1973) The influence of photoperiod and melatonin on testis size, body weight, and pelage color in the Djungarian hamster (*Phodopus sungorus*). J. Comp. Physiol. 85:267-282.

Honma S, Honma K, Shirakawa T, and Hiroshige T (1988) Rhythms in behaviors, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. Physiol. Behav. 44:247-255.

Jefimow M, Wojciechowski M, Masuda A and Oishi T (2004) Correaltion between torpor frequency and capacity for non-shivering thermogenesis in the Siberian hamster (*Phodopus sungorus*). J. Thermal Biol. 29:641-647.

Masuda A and Oishi T (1988) Effects of photoperiod and temperature on body weight, food intake, food storage, and pelage color in the Djungarian hamster, *Phodopus sungorus*. J. Exp. Zool. 248:133-139.

Masuda A and Oishi T (1989) Effects of photoperiod, temperature and testosterone-treatment on plasma T3 and T4 levels in the Djungarian hamster, *Phodopus sungorus*. Experienia 45:102-103.

Masuda A and Oishi T (1995) Effects of restricted feeding on the light-induced body weight change and locomotor activity in the Djungarian hamster. Physiol. Behav. 58:153-159.

Paul MJ, Kauffman AS and Zucker I (2004) Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. J. Biol. Rhythms 19:226-237.

Prendergast BJ and Freeman DA (1999) Pineal-independent regulation of photo-nonresponsiveness in the Siberian hamster (*Phodopus sungorus*). J. Biol. Rhythms 14:62-71.

Puchalski W and Lynch GR (1986) Evidence for differences in the circadian organization of hamsters exposed to short-day photoperiod. J. Comp. Physiol. 159A:7-11.

Refinetti R (1999) Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species. Am. J. Physiol. 277:1493-1500.

Ruby NF, Ibuka N, Barnes BM, and Zucker I (1989) Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. Am. J. Physiol. 257:R210-R215.

Ruby NF, Joshi N and Heller HC (1998) Phase shift magnitude and direction determine whether Siberian hamsters reentrain to the photocycle. J. Biol. Rhythms 13:506-517.

Ruf T and Heldmaier G (1992a) Reduced locomotor activity following daily torpor in the Djungarian hamster recovery from hypothermia? Naturwissenshaften 79:574-575.

Ruf T and Heldmaier G (1992b) The impact of daily torpor on energy requirements in Djungarian hamster, *Phodopus sungorus*. Physiol. Zool. 65:994-1010.

Steinlechner S, Stieglitz A, Ruf T (2002) Djungarian hamsters: A species with a labile circadian pacemaker? Arrhythmicity under a light-dark cycle induced by short light pulses. J. Biol. Rhythms 17: 248-258.

Tannenbaum MG and Pivorum EB (1987) Differential effects of food restriction on the induction of daily torpor in *Peromyscus*

maniculatus and *Peromyscus leucopus*.　J. Thermal Biol. 12:159-162.

Tsutsui K, Kawashima S, Masuda A and Oishi T (1988)　Effects of photoperiod and temperature on the binding of follicle-stimulating hormone (FSH) to testicular preparations and plasma FSH concentration in the Djungarian hamster, Phodopus sungorus.　Endocrinol. 122:1094-1102.

Tsutsui K, Kawashima S, Masuda A and Oishi T (1989)　Changes in the testicular binding of luteinizing hormone and plasma testosterone concentrations in the Djungarian hamster subjected to different photoperiods and temperatures and effects of long-term testosterone treatment on the binding. J. Exp. Zool. 251:91-100.

Tucker VA (1962) Diurnal torpidity in the California pocket mouse.　Science 136: 380-381.

Widman DR, and Timberlake W (1995) Two possible determinants of the timing of daily episodes of behavior in rats.　Physiol. Behav. 58:1227-1236.

ジャンガリアンハムスター（*Phodopus sungorus*）の体温リズムと歩行活動リズムにおける日内休眠と関連した概日およびウルトラジアン成分の分離発現

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　ジャンガリアンハムスターは、冬季において日中体温を約10‐20℃減少させる日内休眠を示す。今回、休眠と非休眠の状態において体温および歩行活動の概日およびウルトラジアンリズムの特徴を明らかにすることを試みた。実験の前に、雄の成体ハムスターを長日・高温（LP-HT）においた、その後、動物を短日・低温（SP-LT）に移し、約6ヶ月の間、この条件のもとにおいた。実験中、体重と摂食量をモニターした。歩行活動と体温のリズムをテレメーターシステムにより3分から5分間隔で記録した。SP-LTに対する反応において二つのタイプが認められた、すなわち、体温を下げて日内休眠を示すタイプと体温を下げずに日内休眠を示さないタイプである。非休眠個体は実験期間中ずっと体温と歩行活動において概日リズムを示した。一方、休眠個体は、日内休眠を示す期間、体温は概日リズムを示し、歩行活動は概日リズムとウルトラジアンリズムの両方を示した。しかしながら、非休眠時における休眠個体は、体温リズムは概日リズムとウルトラジアンリズムの両方の成分を示したが、歩行活動リズムにおいては、ウルトラジアンリズムのみを示した。この概日リズムとウルトラジアンリズムの分離発現の機能的意味について検討した。

キーワード：日内休眠、歩行活動、体温、概日リズム、ウルトラジアンリズム