Research article

Protein malnutrition and aging affects entraining and intensity of locomotor activity and body temperature circadian rhythms in rats

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Independently, chronic protein malnutrition and aging have been shown to affect locomotor activity (LA) and body temperature (BT) rhythms in mammals. The objective of the present study was to ascertain the combined effects of these two factors by examining period, entrainment and other circadian parameters between LA and BT rhythms. Chronic protein malnourished (PM) and well-nourished (WN) male Sprague–Dawley rats (550–590 days of age) were implanted with activity-temperature intraperitoneal radio transmitters (Mini Mitter) and exposed to different lighting protocols during at least 10 days – light-dark cycles (LD 12:12), constant darkness (DD), skeleton photoperiod (SP) and again LD. Results indicate that parametric entrainment, achieved by means of complete photoperiod, is not negatively affected in malnourished rats; however, it is affected under non-parametric entrainment like SP. A different free running period between the LA and BT circadian rhythms was detected for well-nourished and malnourished aged rats.

Keywords: locomotor activity, body temperature, protein malnutrition, aging, circadian rhythms

Introduction

Circadian rhythms enable the organism to fit physiological and behavioral mechanisms to the external cycles determined by geophysical changes to appropriate times of the day. In mammals they are mainly controlled by the suprachiasmatic nucleus (SCN), and their physiology depends on the interaction among SCN and a diversity of peripheral oscillators thorough neuroendocrine signals.[1] Central and peripheral oscillators differ in their phasing and their response to external cues and aging has a disrupting impact in such way that notable changes on amplitude and period length of circadian rhythms may produce an internal desynchronization (for review, see Van Someren[2]). Aging seems to affect mainly interactions among circadian oscillators, perhaps attenuating the SCN ability to drive damped oscillators in the periphery,[3] and also produces changes in circadian gene expression.[4,5] It has been shown that the circadian rhythm of neuropeptide synthesis and release in the SCN is modified by aging in primates, in which differential regulation of the two main peptidergic cell populations affect the ability of the SCN to transmit rhythmic information to other neural target sites.[6]

On the other hand, nutrition plays a critically important role in the development and functional

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organization of the nervous system. It is known that malnutrition adversely affects nervous system maturation and functional development.[7,8] In rats, chronic low protein malnutrition alters the circadian system by producing a lack of coupling among oscillators, probably due to the neuronal changes observed in SCN.[9] When aging and protein malnutrition are combined in adults, together there are significant structural and functional changes within the central nervous system. For example, phase-shift advances of the spontaneous locomotor rhythm alter the rest/activity ratio in adults, possibly due to alterations in entrainment to the light-dark cycle, and/or in the coupling force of the circadian oscillators.[9,10] Nowadays, the long-lasting effects of protein malnutrition on circadian functions are seldom investigated. The present work contributes with information about the combined effects of both aging and malnutrition. In developing countries, both factors are often present simultaneously in the human population that experiences not only advancing years but also the effects of malnutrition over a life-time.

The present work studies the effects of protein malnutrition and the concomitant effects of aging on circadian rhythms of body temperature (BT) and locomotor activity (LA). Results obtained indicate that parametric entrainment, achieved by means of complete photoperiod, is not negatively affected in malnourished rats; however, it is affected under non-parametric entrainment which consists of skeleton photoperiods. A different, free-running period was detected in both aged well-nourished and aged malnourished rats between the LA and BT circadian rhythms.

Materials and methods

Animals

Sprague–Dawley rats, originally obtained from several breeders, were born and raised at the Instituto de Neurobiologia vivarium. Two groups of 10 nuliparous female rats (250 g) each were fed on one of the two diets – laboratory diet (casein chow standard feeding; Purina, USA) or low-protein diet (casein 6%; Teklad, WI, USA) – 5 weeks before mating and thorough gestation, lactation, and until assayed. During mating, the female rats were caged with males in a proportion of 2:1 for the period of a week or when sperm was present on vaginal smears. Mating males were fed with the diet corresponding to the assigned females. When parturition occurred, the litters born on the same day were culled, sexed and standardized to 8 pups each (2 females, 6 males) and after lactation, in order to avoid litter effects, one rat of each litter by condition was taken to group 10 well-nourished (WN) and 10 protein malnourished (PM) male rats, which were fed *ad libitum*; these rats were allowed to grow for about 550 days before testing according to their diet group.

Experimental procedure

All surgery was performed during the subjective day of the animals. Paraffin-coated radio transmitters (Model VM-FH Mini Mitter; Sun River, OR, USA) were abdominally implanted in chloral-hydrate anesthetized rats (4.7 mg/kg). After surgery, each rat was individually placed in a Plexiglas cage until recovery. Each cage was provided with control or protein-deficient chow and water *ad libitum*.

BT and LA data were collected in 10-min bins by means of the Vital View data acquisition system. Each animal, in its plastic cage, was placed on a radio receiver base (Model RA-1010). All cages were placed inside a sound attenuated room. Environmental temperature was maintained in 24–25°C and 40–50% humidity. LA and BT were recorded under different consecutive light protocols over at least 2 weeks for each condition. First, animals were placed in 12:12 light–dark (LD) cycles, lights on 07:00 lights off 19:00 h. Light at the bottom of each cage (100–200 lx) was provided by a white fluorescent lamp controlled by a domestic timer. Then, data were collected under conditions of constant darkness (DD) for at least two more weeks in order to study free running rhythms. While in this condition, animal maintenance was performed by using dim red light (1–3 lx) when needed and at different times of day. After DD, the skeleton photoperiod (SP), consisting of two pulses of light of 30 min each were applied at the time of artificial dusk and dawn (7:00–7:30 h and 18:30–19:00 h). Finally, original LD conditions were re-assayed.

Statistical analysis

Recordings over 7–10 days of steady-state rhythms of BT and LA from each condition were plotted and analyzed by χ^2 periodograms[11] by means of TAU software (Mini Mitter). Periodicity was calculated using a periodogram at 0–30-h intervals. Periodograms with spikes above the confidence interval ($P < 0.05$) were considered rhythmic. The average waveform for each experiment was fitted to the resultant period to obtain the maximal and mean daily activity. During LD and SP, entrainment was evaluated by using transients and the phase differences between activity onset and lights-on during the steady state of the rhythm. Results obtained under the different protocols were analyzed

Table 1 **Percentage of rats presenting entrainment (E), non-entrainment (NE), free-running rhythm (FR) and arrhythmia (A) during different lighting protocols for body temperature and locomotor activity rhythms**

	Light-dark			Constant darkness		Skeleton photoperiod			Light-dark		
	E	NE	A	FR	A	E	NE	A	E	NE	A
Body temperature											
Well-nourished (%)	50	30	20	60	40	10	40	60	30	30	40
Protein malnourished (%)	80	10	10	90	10	30	50	20	50	50	0
Locomotor activity											
Well-nourished (%)	50	0	50	50	50	10	0	90	50	10	40
Protein malnourished (%)	100	0	0	80	20	40	0	60	50	20	30

by means of a non-paired Student's *t*-test and significant differences were considered when $P < 0.05$. Results are indicated as averages (± SE) unless indicated.

Results

During LD, a higher percentage of rhythmicity in both BT and LA was found for malnourished compared to well-nourished animals. Also, BT significant free running rhythms in DD and entrainment to SP were found more often in malnourished rats. The percentage of entrainment, free running rhythms or lack of rhythmicity in BT and LA both in WN and PM animals are shown in Table 1.

Representative actograms of LA (left) and BT (right) of a rat from the WN group are shown in Figure 1. Disperse bouts of activity were obtained along the entire recording; however, nocturnal activity can still be distinguished. Body temperature rises before the LA increases after lights off (Figure 3). During DD, poor rhythmicity can be observed in both BT and LA that fades by the fifth day for this condition. When exposed to SP, LA bouts are organized close to each light pulse and BT has a peak that follows the evening pulse. When exposed to LD, LA regroups after several days to the nocturnal phase, while body temperature fades outwith the clipping limits of the actogram (37–38.5°C).

Figure 2 shows two representative actograms of LA (left) and BT (right) from a rat of the PM group. Activity is dispersed throughout the entire data recording; however, bouts are clearer during the night than during

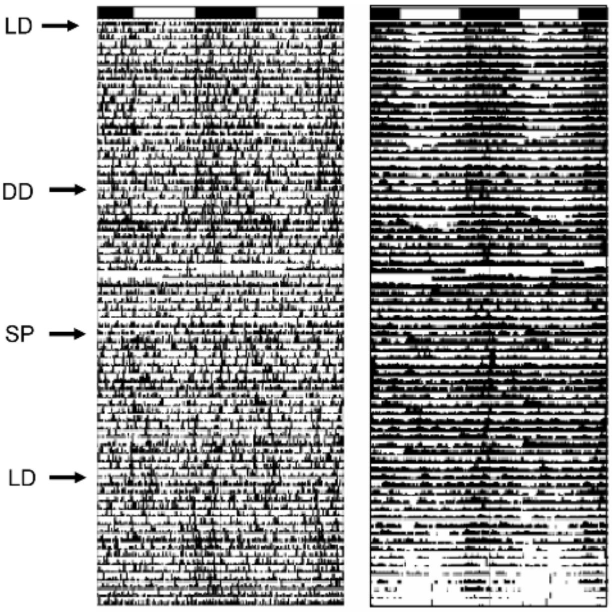

Figure 1 **Double plotted 24 h-actograms of locomotor activity (left) and body temperature (right) of a rat from the well-nourished group. Light conditions are indicated on the left of the figure**

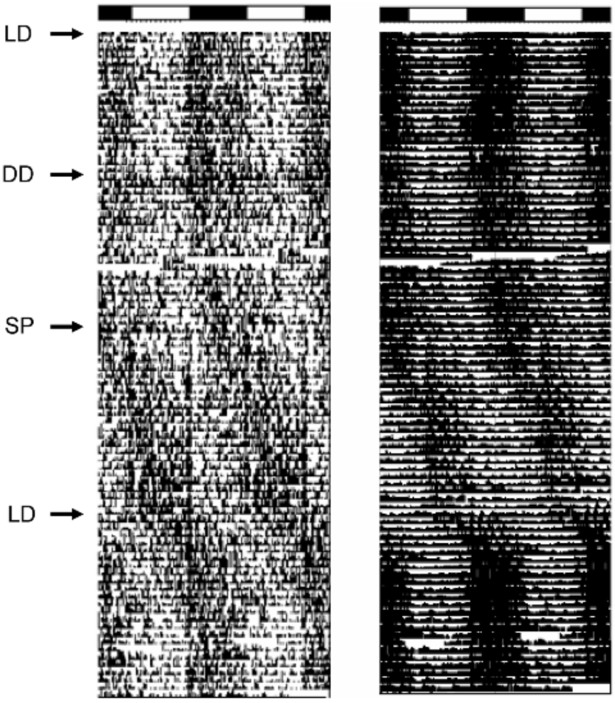

Figure 2 **Double plotted 24 h-actograms of locomotor activity (left) and body temperature (right) of a rat from the prenatally malnourished group. Light conditions are indicated on the left of the figure. When submitted to skeleton photoperiod (SP), the rhythms free run and entrain about 10 days before the switch to the LD cycle. Temperature values are plotted in a scale of 37–38.5°C**

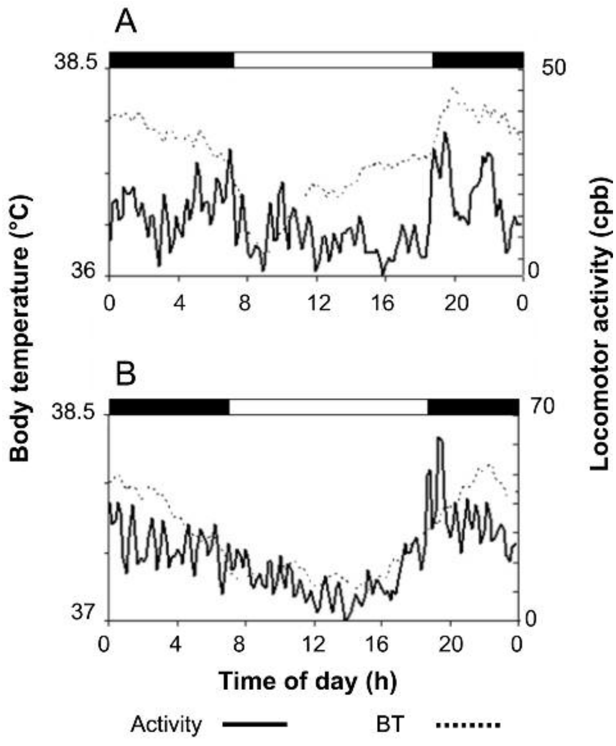

Figure 3 Average waveform of locomotor activity and body temperature of a well-nourished rat (upper graph) and a protein malnourished rat (lower graph). BT increases in the well-nourished rat before the main onset of activity; in the protein malnourished rat, BT rises almost in phase with locomotor activity (CPB, counts per bin)

the day in LD. During the skeleton photoperiod (SP), both rhythms free run to entrain in the middle of the subjective day. When shifted again to LD, LA and BT re-entrain the rhythm with a nocturnal phase. During LD, both LA and BT differences are clearer than when subjected to DD or SP.

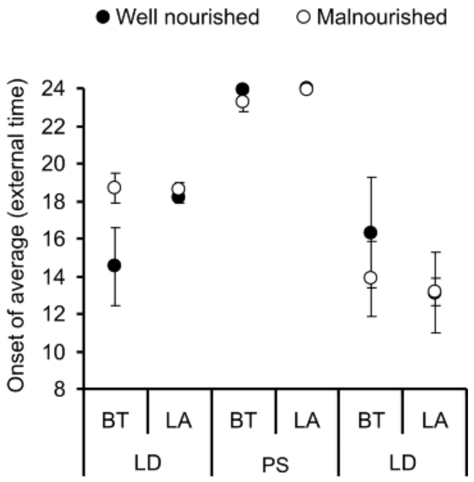

Figure 4 Mean values (± SE) for the onset average amplitude for either body temperature rhythms (BT) or activity rhythms (LA) under different light conditions. Only data that displayed a periodogram of 24 ± 1 h are shown

The average waveforms of LA (straight lines) and BT (dotted lines) of two representative animals held in LD illustrate that body temperature rises before the main phase of LA in WN rats, while BT arises close to LA in PM rats (see Fig. 3).

Phase relationship between BT and LA

During initial LD, PM rats that entrained the BT rhythm to the zeitgeber, presented the average onset at 18.66 ± 0.8 h. Well-nourished rats that entrained BT rhythm displayed such onset earlier that PM group (14.54 ± 2.07 h; $P < 0.05$) indicating that BT arises before the onset of LA (Fig. 4). In subsequent entraining conditions, even non-entrained animals during free running displayed a close relationship between BT and

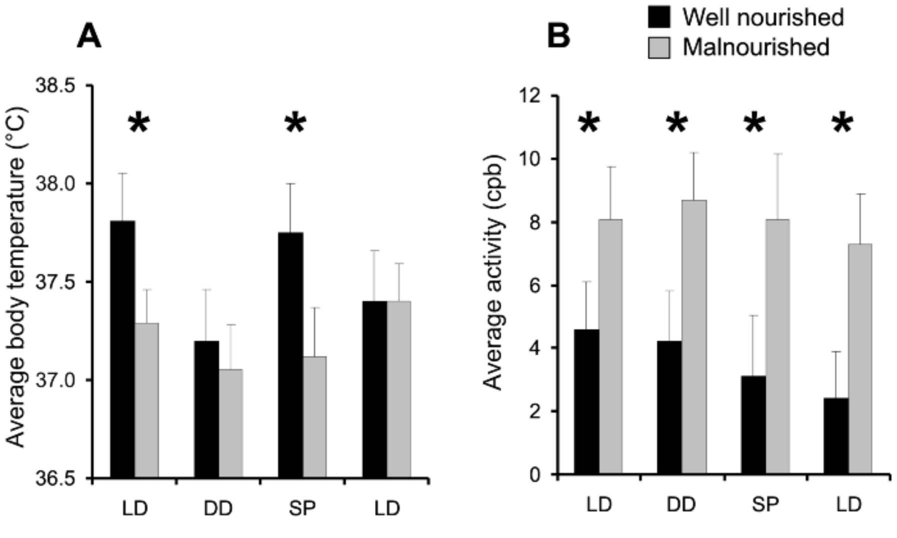

Figure 5 Average values (± SE) for body temperature (A) and locomotor activity in counts per bin (cpb) in (B). Even though body temperature was higher in controls (black bars), protein malnourished rats were more active (gray bars)

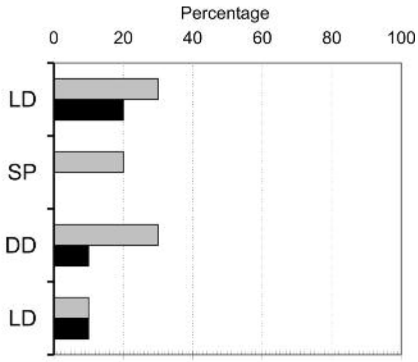

Figure 6 **Percentage of animals in which period detected by periodogram were different in the same light condition between BT and LA. Black bars represent the well-nourished group while gray bars represent the protein malnourished group**

LA. During the last LD, no differences were found; however, data were more disperse between groups.

Average amplitudes

Figure 5A illustrates the average body temperature between WN and PM. Differences were detected during initial LD and SP photic conditions, where controls displayed higher values. Average activity (Fig. 5B) also was different between WN and PM rats. Higher values of activity were noted in PM ($P < 0.05$).

Period variability

Differences in period (at least 0.2 h between each variable) between BT and LA were repeatedly detected using periodogram after the initial LD conditions in PM rats. Periods for the WN group showed more stability (see Fig. 6).

Discussion

The complexity of the circadian system and the internal coupling are widely affected during aging since, in the SCN, the number of functioning neurons decreases its coupling as well as their ability to drive a strong output to different organs.[12] The effects of aging on circadian rhythms in rats lead mainly to disrupted internal circadian organization between SCN and peripheral oscillators,[3] as well as the ability to resynchronize peripheral oscillators to a shifted LD regimen.[13] The lack of co-ordination of this internal timing in rodents may result in the typical behavioral effects observed, such as alteration of the phase angle entrainment to the LD cycles and phase shifting to light pulses and transient cycles during re-entrainment.[14]

In the present work, we observed that aged, well-nourished rats display typical behavior mentioned above, and also noted that rhythmicity is statistically absent in some well-nourished rats both in LA and BT. In contrast, a lower percentage of malnourished arrhythmic rats was observed (Table 1), indicating that the mechanisms underlying internal desynchronization during aging should be attenuated in malnourished animals.

Malnutrition cause a body mass decrease in Sprague–Dawley rats;[15] however, malnourished, aged rats display a larger amount of activity than well-nourished animals (Fig. 5B). It is known that malnutrition and low body weight appear to mediate some of the mortality-reducing effects of calorie restriction in rats.[16] Hyperactivity in PM rats seems to be related also with malnutrition itself, consistent with previous observations;[17] this effect seems to occur in different species.[18] The increased LA may explain why malnourished, aged rats have better rhythms than well-nourished rats and may be related to the fact that physical activity in elderly humans improves the ability to perform better phase shifts in a LD regimen[19] and achieve better sleep.[20]

The PM rats displayed a larger percentage of adjustment to LD conditions. This observation confirm previous results in young and adult rats,[9] in which malnourished rats takes about half the time required for well-nourished animals to entrain fully to the complete photoperiod. That aged, well-nourished rats displayed poor entrainment to SP together with the fact that a larger number of malnourished animals entrained to the skeleton photoperiod than well-nourished rats, reinforces the observation that a non-significant difference seems to occur in the circadian photic pathway during prenatal malnutrition.[21] It is possible that differences observed in well-nourished compared with PM rats might be due to the degenerative effects of aging on circadian photic pathways, being stronger in well-nourished animals; nevertheless, malnourished rats that entrain to SP take about 10–15 cycles to phase lock any of the pulses (Fig. 2) as happens in younger animals.[9]

Malnourished, aged rats presented an average body temperature lower than well-nourished animals when exposed to LD and SP. Previous studies using PM rats showed a decrease in brain temperature in aged compared with young rats[17] and body temperature usually is lower in aged rats.[22] In the present study, we found that body temperature may change with light conditions. A similar response has been observed in Sprague–Dawley rats indicating that chronic under-nutrition may produce lower average body temperature and affect the ability to recover from common environmental stressors.[23] Finding these differences only during entrainment conditions may indicate that, in aged animals, changing photic environmental conditions may be a stressor that induces differences in thermoregulating responses. Body temperature in humans also varies with age; in comparison with adults, it is poorly developed in the neonate and deteriorates in the aged.[24]

A phase advance of increased mean body temperature as well as locomotor activity under LD conditions existed in both well-nourished and PM rats (Figs 3 and 4); however, such advance was shorter in BT of PM rats. Aging produces a phase advance of LA and BT in old Wistar rats submitted to LD conditions[22] and malnourished adult rats display an advanced rise in locomotor activity when compared with well-nourished rats.[10] Here, we noticed that BT phase in well-nourished rats demonstrated a larger variability than PM animals. Such variability between activity and temperature in well-nourished rats is larger than that observed in the relationship between cortical temperature and locomotor activity, in which well-nourished, old rats had larger variability in acrophase than malnourished old rats.[17] The variability of onset of LA and BT to LD observed in the latter conditions is consistent with the fact that old, malnourished rats display a bimodal rhythm previously observed in younger rats.[9]

When the period values of BT and LA rhythms are compared, PM rats more frequently presented different period values in the same organism. Such differences in old rats were also observed when periodicities of cortical temperature and locomotor activity were compared in old rats.[17] This result may be interpreted as a consequence of the weak circadian oscillation present in the PM group that might affect the period calculation, or the possibility that there is an internal desynchronization between LA and BT. Since aging has an effect of internal coupling of oscillators,[3,4] this possibility seems more plausible than other reports in malnourished Sprague–Dawley rats in which force of coupling among internal oscillators has been considered as due to the circadian effects of malnutrition.[9,10,17]

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References

1. Meijer JH. Photic entrainment of mammals. In: Takahashi J, Turek FW, Moore RY. (eds) *Handbook of behavioral neurobiology. 12 Circadian clocks*. New York: Kluwer, 2001; 183–210.
2. Van Someren EJ. More than a marker: interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. *Chronobiol Int* 2000; **17**: 313–54.
3. Yamazaki S, Straume M, Tei H, Sakaki Y, Menaker M, Block GD. Effects of aging on central and peripheral mammalian clocks. *Proc Natl Acad Sci USA* 2002; **99**: 10801–10806.
4. Claustrat F, Foumier I, Geelen G, Brun J, Corman B, Claustrat B. Aging and circadian clock gene expression in peripheral tissues in rats. *Pathol Biol (Paris)* 2005; **53**: 257–260.
5. Asai M, Yoshinobu Y, Kaneko S *et al.* Circadian profile of *Per* gene mRNA expression in the suprachiasmatic nucleus, paraventricular nucleus, and pineal body of aged rats. *J Neurosci Res* 2001; **66**: 1133–1139.
6. Cayetanot F, Bentivoglio M, Aujard F. Arginine-vasopressin and vasointestinal polypeptide rhythms in the suprachiasmatic nucleus of the mouse lemur reveal aging-related alterations of circadian pacemaker neurons in a non-human primate. *Eur J Neurosci* 2005; **22**: 902–910.
7. Morgane PJ, Austin-LaFrance RJ, Brozino JD *et al.* Prenatal malnutrition and development of the brain. *Neurosci Biobehav* 1993; **17**: 91–128.
8. Duran P, Galván A, Granados L, Aguilar-Roblero R, Cintra L. Effects of protein malnutrition on vigilance state and their circadian rhythms in 30-day old rats submitted total sleep deprivation. *Nutr Neurosci* 1999; **2**: 127–138.
9. Aguilar-Roblero R, Salazar-Juarez A, Rojas-Castaneda J, Escobar C, Cintra L. Organization of circadian rhythmicity and suprachiasmatic nuclei in malnourished rats. *Am J Physiol* 1997; **273**: R1321–R1331.
10. Duran P, Cintra L, Galler JR, Tonkiss J. Prenatal protein malnutrition induces a phase shift advance of the spontaneous locomotor rhythm and alters the rest/activity ratio in adult rats *Nutr Neurosci* 2005; **8**: 167–172.
11. Sokolove PG, Bushell WN. The chi square periodogram: its utility for analysis in circadian rhythms. *J Theor Biol* 1978; **72**: 131–160.
12. Weinert D. Age-dependent changes of the circadian system. *Chronobiol Int* 2000; **17**: 261–283.
13. Davidson AJ, Yamazaki S, Arble DM, Menaker M, Block GD. Resetting of central and peripheral circadian oscillators in aged rats. *Neurobiol Aging* 2006; doi:10.1016/j.neurobiolaging.2006.10.018.
14. Turek FW, Penev P, Zhang Y, Van Reeth O, Takahashi JS, Zee P Alterations in the circadian system in advanced age. *Ciba Found Symp* 1995; **183**: 212–226, discussion 226–234.
15. Cintra L, Diaz-Cintra S, Galván A, Morgane P. Circadian rhythm of sleep in normal and undernourished rats. *Bol Est Med Biol* 1988; **36**: 3–17.
16. Wang C, Weindruch R, Fernández JR, Coffey CS, Patel P, Allison DB. Caloric restriction and body weight independently affect longevity in Wistar rats. *Int J Obes Relat Metab Disord* 2004; **28**: 357–362.
17. Castanon-Cervantes O, Cintra L. Circadian rhythms of occipital-cortex temperature and motor activity in young and old rats under chronic protein malnutrition. *Nutr Neurosci* 2002; **5**: 279–286.
18. Weed JL, Lane MA, Roth GS, Speer DL, Ingram DK. Activity measures in rhesus monkeys on long-term calorie restriction. *Physiol Behav* 1997; **62**: 97–103.
19. Baehr EK, Charmane IE, Revelle WS, Olson LF, Zee PC. Circadian phase shifting effects of nocturnal exercise in older compared with young adults. *Am J Physiol* 2003; **284**: R1542–R1550.
20. Ceolim MF, Menna-Barreto L. Sleep/wake cycle and physical activity in healthy elderly people. *Sleep Res Online* 2000; **3**: 87–95.
21. Vilela MCR, Mendonca JEF, Bittencourt H *et al.* Differential vulnerability of the rat retina, suprachiasmatic nucleus and intergeniculate leaflet to malnutrition induced during brain development. *Brain Res Bull* 2005; **64**: 395–408.
22. Benstaali C, Bogdan A, Touitou Y. Effect of a short photoperiod on circadian rhythms of body temperature and motor activity in old rats. *Pflügers Arch* 2002; **444**: 73–79.
23. Campbell BA, Richardson R. Effect of chronic undernutrition on susceptibility to cold stress in young adult and aged rats. *Mech Ageing Dev* 1988; **44**: 193–202.
24. Weinert D, Waterhouse J. The circadian rhythm of core temperature: effects of physical activity and aging. *Physiol Behav* 2007; **90**: 246–256.