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B. Nuesslein-Hildesheim · K. Imai-Matsumura
H. Döring · I. Schmidt

Pronounced juvenile circadian core temperature rhythms exist in several strains of rats but not in rabbits

Abstract Torpor-like circadian variations of core temperature are well documented for suckling-age Zucker rat pups. To determine (1) whether this juvenile circadian rhythm is as strongly expressed in other rat strains, and (2) whether a similar rhythm is expressed in rabbit pups, we recorded core temperature and metabolic rate of artificially reared pups. Wistar, Brown Norway, and Long Evans pups were studied for 30 h under moderate cold loads (ambient temperature = 28 °C) when 9–11 days old, i.e. at the age and ambient temperature for which the rhythm has been most thoroughly characterized in Zucker rats. Chinchilla bastard rabbit pups were studied under similar conditions when they were 3–8 days old, the youngest age at which the rhythm can be easily detected in rats. Rat pups of each strain showed clear circadian rhythms with sharp decreases of core temperature and metabolic rate in subjective morning. Core temperature amplitudes were in the order Wistar < Brown Norway < Zucker < Long Evans strain. In contrast, the rabbit pups maintained stable high levels of core temperature and metabolic rate throughout the day. A torpor-like decrease of core temperature in the morning is thus not a peculiarity of the Zucker rat strain but also occurs in other pigmented rat strains, whereas rabbit pups at a similar developmental stage do not show a circadian core temperature rhythm.

Key words Strain differences · Nursing behaviour · Developmental changes · Thermoregulation · Rat pups

B. Nuesslein-Hildesheim · K. Imai-Matsumura[1]
H. Döring · I. Schmidt (✉)
Max-Planck-Institut für Physiologische und Klinische Forschung,
W. G. Kerckhoff-Institut, Parkstraße 1, D-61231 Bad Nauheim,
Germany

Present address:
[1]Faculty of Education, Shiga University, Otsu, Japan

Abbreviations bm body mass · MR metabolic rate · RH relative air humidity · T_a ambient temperature · T_c core temperture

Introduction

Although the altricial rat pup is born neurologically and functionally rather immature (Nedergaard et al. 1986; Romijn et al. 1991), artificially reared Zucker (Nuesslein and Schmidt 1990) and Wistar albino rat pups (Mumm et al. 1990) both develop an endogenous circadian rhythm of T_c and MR during the first week of life. The amplitude of the juvenile T_c rhythm in Wistar pups is, however, only half that observed in genetically lean (Fa/-) Zucker rats under identical conditions (Mumm et al. 1989, 1990). The first aim of the work reported here was to find out whether any other rat strain also shows juvenile circadian rhythms with T_c amplitudes as large as those occurring in moderately cold-loaded Zucker rat pups. As the juvenile T_c rhythm in Zucker rats is most strongly expressed in the second week of life (Nuesslein and Schmidt 1990), we recorded T_c and MR of 9- to 11-day-old Wistar, Brown Norway and Long Evans rats under the same experimental conditions as previously used for Zucker rats.

Wild as well as domestic rabbits experience an even more pronounced diurnal cycle of maternal care than rats do. Whereas rat dams visit the litters repeatedly, though less frequently during the night than during the day, the rabbit doe comes to the litter only once a day for 3–4 min, usually at the end of dark phase (Ader and Grota 1970; Hudson and Distel 1989). One to two hours before this short nursing period, during which 1-week-old rabbit pups ingest one third of their body weight in milk, the pups prepare themselves for their mother's arrival by uncovering themselves from the nest material and huddling tightly together (Hudson and Distel 1989). Five-day-old litters isolated from their mother for 48 h uncover themselves at the usual

time even on the second day without food (Hudson and Distel 1982), thus indicating that this prenursing activity might reflect an endogenous rhythm.

Our second aim was therefore to find out whether artificially reared rabbit pups do develop an endogenous circadian rhythm of T_c and MR at a similar developmental stage as rats. To meaningfully compare the thermoregulatory activity of pups of very different sizes, the degree of cold loading should not be measured in terms of T_a (Schmidt et al. 1987), because the rate of heat loss at the same T_a is much greater in smaller pups. Because rabbit pups, like rat pups, can increase their MR under cold load to about 250% of their thermoneutral MR (Hull 1965; Spiers and Adair 1986), the degree of cold loading is better compared in terms of the proportion by which MR increases above its thermoneutral level (Körtner et al. 1993). Because 1-week-old rat pups express strong circadian rhythms under ambient conditions resulting in their average daily MR being 50–100% above its thermoneutral level (Mumm et al. 1989; Nuesslein and Schmidt 1990), we studied the rabbit pups at a similar degree of metabolic activity.

Materials and methods

Animals

Our breeding stocks of Wistar, Brown Norway and Long Evans rats (purchased from Charles River Wiga, Sulzfeld, FRG) were maintained at 22 °C and 55% RH in rooms with lights on from 0700 to 1900 hours (Wistar and Long Evans) or to 2100 hours (Brown Norway). Water and food pellets were provided ad libitum. Pups were reared by their mothers in the colonies until artificial rearing started at 9 or 10 days of age (day of birth = day 0). At this age the average bm of the Wistar pups (19.5 ± 0.4 g, $n = 25$) and Long Evans pups (20.4 ± 0.4 g, $n = 29$) was significantly greater ($P < 0.05$) than that of the Brown Norway pups (17.0 ± 0.5 g, $n = 14$).

Pregnant Chinchilla bastard does (Oryctolagus cuniculus) were obtained from SAVO Ivanovas (Kisslegg, FRG) 2 weeks before delivery and placed in cages with attached nestboxes in a room with lights on from 0700 to 1900 hours. Nursing events were detected by a photoelectric gate 15 cm above the bottom of the nestbox. The pups were mother reared at 20 °C and 65% RH until they were 3–4 days old and weighed 83 ± 2 g ($n = 15$).

Procedure

Artificial rearing of the rat pups was carried out as previously described for Zucker rats (Mumm et al. 1989; Kaul et al. 1990; Nuesslein and Schmidt 1990), except that the pups were studied only for 30 h. The experiments were carried out in a climatic chamber at a T_a of 28 °C and 60% RH. Each pup was kept in continuous dim light in its own plastic container floating in a temperature-controlled water bath (Mumm et al. 1989). Multichannel infusion pumps supplied synthetic rat milk (Messer et al. 1969) at about 3.8 ml · day^{-1} via a chronic oesophageal catheter made from a 3.5-cm length of soft Silastic tubing (Dow Corning 602-105) (Nuesslein and Schmidt 1990).

The rabbit pups were artificially reared following the same experimental protocol used for the rat pups in our first studies (Mumm et al. 1989, 1990). Pups were maintained for 3–5 days in continuous dim light and tended at 16-h intervals (at 0600 and 2200 or 1400 hours). Because the rabbits weighed about ten times as much as rats of the same age, longer (6 cm) and more robust (Dow Corning 602-155) catheters were used. Each pup was singly placed in a large plastic container and maintained at a lower T_a (26–27 °C) than equally aged rat pups (Mumm et al. 1989, 1990; Nuesslein and Schmidt 1990) to drive a similar 60–80% increase of their average daily MR above its thermoneutral level of 6.6 W · kg^{-1} (Hull 1965). The rate at which we infused synthetic rabbit milk (Schley 1980) was increased from about 16 ml · day^{-1} for 3-day-old pups to about 25 ml · day^{-1} for 7-day-old pups, according to body size and degree of stomach filling. In one additional experiment starting in the morning, five 6-day-old rabbit pups were maintained at a T_a of 31 °C for 30 h without milk.

Measurements

Temperatures and MR were measured as previously described in detail (Kaul et al. 1990; Nuesslein and Schmidt 1990; Nuesslein et al. 1994). In short, the colonic T_c of as many as 11 pups was continuously recorded from thermocouple probes inserted 2.2 cm beyond the anal sphincter in rat pups and 4.5 cm in rabbit pups. The MR of up to seven animals were successively determined once every 40 min in an open-flow system equipped with a Tylan massflow meter and an Ametek S-3A electrochemical oxygen analyzer. All data were recorded on a multichannel point printer and simultaneously on disk.

Evaluation

For each pup, hourly mean values of T_c were calculated from the data stored every 12 s, starting at 1300 hours on the day of isolation. Assuming the same respiratory quotient (0.8) for the rabbit pups as for the rat pups (Markewicz et al. 1993), each animal's O_2 consumption (STPD) was calculated from the massflow rate and the O_2 content of the dried air continuously aspirated from the animal containers (Withers 1977). The MR was determined from these values by using a conversion coefficient of 0.33 W · min^{-1} · ml O_2^{-1}. Body masses measured for pups with an emptied bladder were linearly extrapolated for calculating mass-specific MR.

Because the characteristics of free-running circadian T_c-rhythms have already been thoroughly analysed in Zucker rat pups (Nuesslein and Schmidt 1990), we limited this study to the strain differences of the daily extreme values in the first 30 h following isolation from the mother. As in our earlier study (Nuesslein and Schmidt 1990), the "daily minimum" of T_c was defined as the lowest hourly mean value, and the "daily maximum" of T_c was defined as the mean of the six highest consecutive hourly mean values. Daily maxima and minima of T_c and their difference (= amplitude of the rhythm) were determined for individual pups. The daily maximum of MR was analogously determined. Mean values ± SE are presented for each strain. Because the variance was not equal in the different groups, the significance ($P < 0.05$) of differences between strains was determined by a one-way ANOVA on ranks (Kruskal-Wallis) followed by pairwise multiple comparisons (Dunn's method).

Chi^2-periodogram analysis (Enright 1965; Sokolove and Bushell 1978) in moderately cold-loaded 9- to 12-day old rat pups had revealed highly significant T_c rhythms (Nuesslein 1993), so the same methods were used to evaluate the T_c recordings of the rabbit pups whose T_c had also been recorded for periods of at least 3 days. Because the construction of χ^2-periodograms requires continuous data sequences, missing hourly mean values (data lost due to thermocouple breakage or interruptions for tending) were filled in conservatively with mean values calculated for the individual over the entire evaluation period. Significance of periodicity was tested at the 0.05 level.
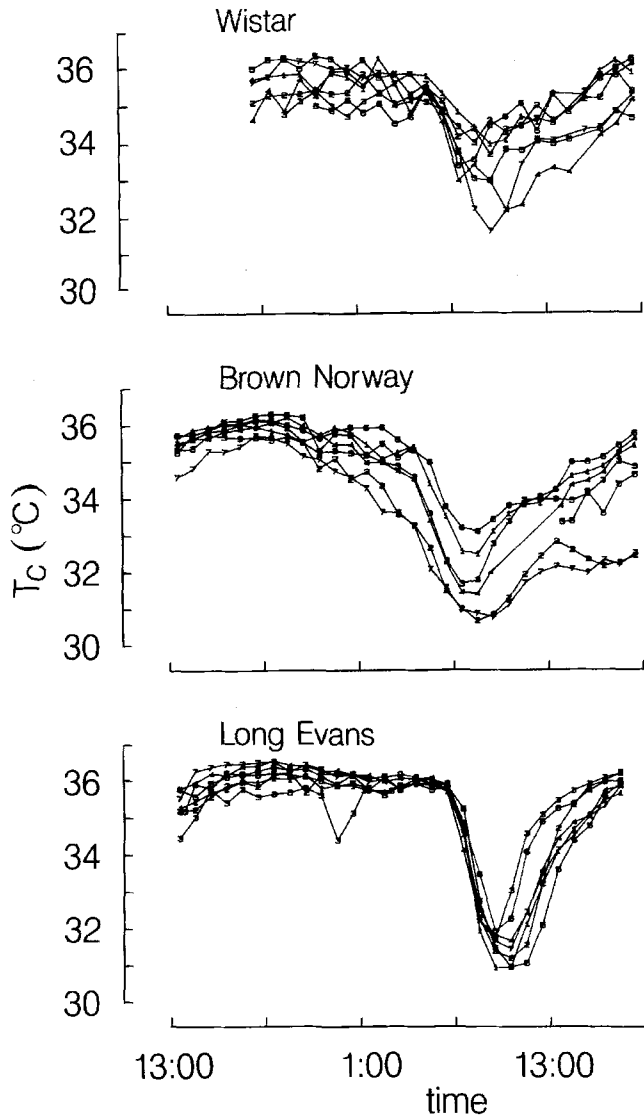
Fig. 1 Hourly mean values of core temperature (T_c) for individual 9- to 11-day-old rats of different strains artificially reared at 28 °C in constant light

Results

T_c and MR of rat pups

The T_c of rat pups from all three strains showed a clear circadian T_c rhythm with a minimum around the time of lights-on in the colony of origin (Fig. 1). The daily maxima of T_c were similar in each strain (about 36 °C) but the daily minima differed between strains. As a result, the average T_c amplitude for the Wistar pups (2.8 ± 0.2 °C, $n = 25$) was slightly smaller than that in Brown Norway pups (3.4 ± 0.2 °C, $n = 13$), and both these values were significantly smaller than that for the Long Evans pups (4.6 ± 0.1 °C, $n = 20$).

To evaluate the possibility of these differences in the T_c amplitude being due to the pups of different strains experiencing different degree of cold loading, we cal-

culated the hourly averages of MR. Like T_c, the MR of all strains decreased sharply at the time at which the dark phase in the colony of origin ended, reaching minimum values of about 12 W · kg^{-1} in the Wistar pups, 10 W · kg^{-1} in the Brown Norway pups, and 6 W · kg^{-1} in the Long Evans pups. But at the time at which the dark phase in the colony of origin began, the MR in all strains was stable at about 80–90% above its thermoneutral level, thus demonstrating that the pups of each strain were subjected to similar degrees of cold loading. Correspondingly, the daily maximum MR (W · kg^{-1}) was identical for the three strains: 17.1 ± 0.4 ($n = 20$) for Wistar pups, 17.9 ± 0.5 ($n = 14$) for Brown Norway pups, and 16.7 ± 0.3 ($n = 14$) for Long Evans pups.

T_c and MR of artificially reared rabbit pups

As Fig. 2 shows for two of the rabbit pups, in none of the 15 continuously fed rabbit pups circadian oscillations of T_c or MR could be visually detected between postnatal days 3 through 7. Their T_c never fell below 37.0 °C, and their MR fluctuated non-periodically around 12 W · kg^{-1} (80% above the thermoneutral MR). Correspondingly, none of the ten pups which were artificially reared for at least 3 days revealed a significant circadian periodicity of T_c in the χ^2-periodogram analysis.

To see if the continuous milk infusion during artificial rearing might have suppressed a diurnal rhythm in these pups which are usually fed only once a day, we additionally studied five 6-day-old rabbit pups for 30 h without milk infusion – and at a higher T_a (31 °C) to avoid a rapid exhaustion of their energy reservoirs. From the nest-box recordings it could be seen that the last nursing had occurred at 0400 hours, and we
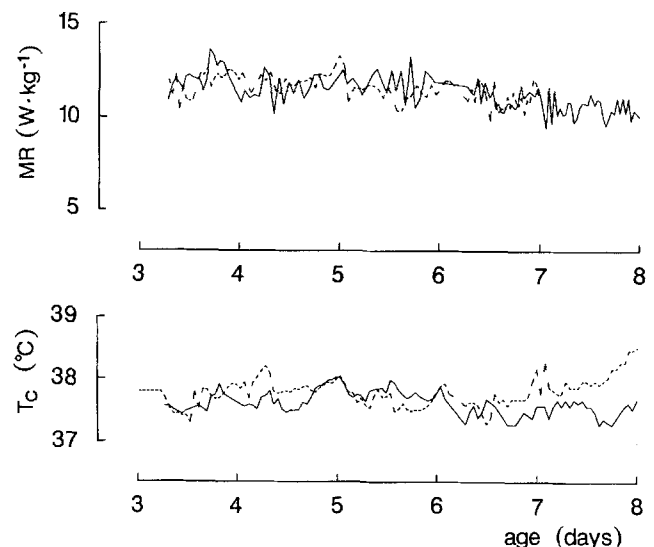

Fig. 2 Examples of metabolic rate (MR) and core temperature (T_c) records for rabbit pups artificially reared for 5 days in constant light under moderate cold loads ($T_a = 26$–27 °C). Note that MR axis does not start at zero.
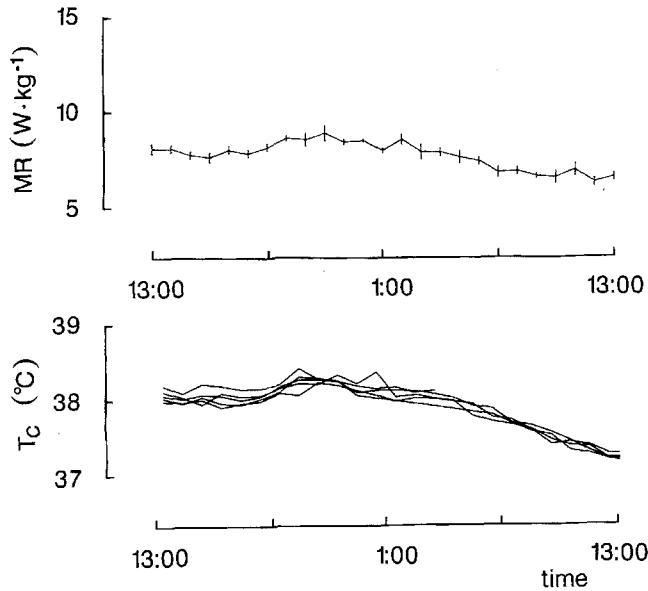
Fig. 3 Average metabolic rate (MR) and individual core temperature (T_c) records for 6-day-old rabbit pups $(n = 5)$, artificially reared for 24 h without food in constant light at 31 °C. Note that MR axis does not start at zero

isolated the pups at 1000 hours. Under these conditions the pups maintained a MR of about 8 W · kg^{-1} (20% above the thermoneutral MR) for 24 h after the last nursing, before MR gradually approached its thermoneutral level (Fig. 3). The individual T_c traces of the five pups showed the same pattern. There was no indication of a diurnal change in T_c or MR at 0400 hours, i.e. 24 h after the last nursing.

Discussion

The main result of this study is that a strongly expressed diurnal T_c rhythm is not a pecularity of the Zucker rat strain but also occurs in two other pigmented rat strains, whereas no trace of a juvenile T_c rhythm could be detected in Chinchilla bastard rabbits. Ten-day-old lean (Fa/-) Zucker rat pups tested under the same conditions as in this study showed diurnal T_c ryhthms with amplitudes of 3.7 ± 0.2 °C $(n = 24)$ (Nuesslein-Hildesheim and Schmidt 1994) and 3.9 ± 0.4 °C $(n = 14)$ (Nuesslein and Schmidt 1990). In Zucker rat pups, the amplitude of the juvenile T_c rhythm in the first 30 h after separation from the mother and after cessation of 24-h cues, is thus slightly larger than that in Brown Norway rats and clearly smaller than that in Long Evans rats. The Wistar rats, and probably also the Sprague-Dawley rats (Spiers 1988), on the other hand, show smaller though still clearly expressed, diurnal rhythms of T_c and MR (Nuesslein 1993). Although suggestive, the number of strains investigated is still too small to confirm that these differences in the amplitude of the juvenile

rhythm are reflecting differences between pigmented and albino rats. However, whatever the cause of these interstrain differences, it is important to note that some commonly studied strains of rats exhibit a juvenile T_c periodicity as pronounced as that thoroughly analysed in lean Zucker rats.

Regardless of the age at which artificial rearing begins, the amplitude of the T_c rhythm of Zucker rat pups maintained at approximately constant cold loads is greatest between 10 and 13 days of age (Nuesslein and Schmidt 1990; Nuesslein 1993). At this age, at the beginning of the light-phase in the colony of origin, the T_c of moderately cold-loaded Zucker rat pups decreases sharply from a plateau value of about 36 °C to a minimum value of about 32 °C (Redlin et al. 1992; Nuesslein-Hildesheim and Schmidt 1994). Under these experimental conditions, during the minimum phase of the T_c rhythm, MR falls to values below the thermoneutral level. Thereafter, MR gradually returns to high levels and reaches a maximum plateau around the beginning of the dark phase in the colony of origin. Whereas the amplitude of the juvenile T_c-rhythm is strongly dependent on T_a and approaches zero at thermoneutral conditions, the time course of the circadian changes of T_c and MR remain similar, and at all but the most severe cold loads the MR during the T_c minimum falls to or even below the thermoneutral level (Nuesslein-Hildesheim and Schmidt 1994).

A juvenile T_c rhythm with features distinctly different from that in the adult (Nuesslein-Hildesheim and Schmidt 1994) has so far been described only for rats. But it was only 10 years ago that – probably due to the arising interest in precise energy balance studies in newborn genetically obese (fa/fa) Zucker rat pups – the juvenile circadian T_c cycle was first noticed in the rat, a species already used for many years in developmental studies (Schmidt et al. 1986; Planche and Joliff 1987). Therefore, it seems likely that appropriate studies will detect juvenile T_c rhythms in other rodent species. However, for the first comparative approach we chose the rabbit as a species belonging to another mammalian order but displaying an even more pronounced diurnal periodicity in its nursing pattern than do rats (Hudson and Distel 1989). Domestic rabbits, such as the Chinchilla bastard, also have a litter size (6–10 pups) similar to that of the laboratory rat. Furthermore, the pups are born in an altricial developmental state similar to that of newborn rats, although their eyes open and their fur emerges some days earlier (Angermann 1972; Harkness and Wagner 1983). Because rat pups artificially reared under cold loads, comparable to that under which our rabbits were investigated, show a clear circadian T_c rhythm from the middle of the first postnatal week to the middle of the third postnatal week (Mumm et al. 1989, 1990; Nuesslein and Schmidt 1990), it is unlikely that methodological short-comings or wrong timing were responsible for the absence of a juvenile T_c rhythm in our rabbit pups.

In speculating, based on these first comparative results, about the biological significance of the torpor-like daily decrease of T_c in juvenile rats, it seems important to consider that the bm of a newborn rat is only one tenth of the 50-g birth mass of a rabbit pup. Like daily torpor, which occurs only in adult rodents weighing less than 50 g (Geiser 1988), the juvenile heterothermia might thus be restricted to species with small pups. And whereas rabbit pups have to depend on themselves for thermoregulation because their mother nurses only a few minutes each day (Hudson and Distel 1989), under similar ambient conditions mothers of 1-week-old rat litters return repeatedly to the nest and spend about 50–80% of the light phase with their young (Schmidt et al. 1986, 1987). From the studies in Zucker rats an estimate of the energy savings due to the juvenile heterothermia can be derived. In isolated 10-day-old artificially reared Zucker rat pups, at a T_a of 28 °C, the average daily energy expenditure was 22% smaller than it would be MR maintained at the maximum value throughout the day (Nuesslein 1993). As shown in earlier studies of Zucker rats (Schmidt et al. 1986, 1987; Mumm et al. 1989; Nuesslein and Schmidt 1990), the cold load experienced by pups huddling at normal rearing temperatures in the nest is similar to that experienced by the isolated artificially reared pups at a T_a of 28 °C. Because mother rats repeatedly return to the nest and spend extended periods with their young during the light phase, the advantage of the morning lability of T_c for mother-reared pups should be even greater than for our artificially reared pups: the time spent in hypothermia is minimalized and the energy necessary for rewarming is taken over by the mother.

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