Characteristics and consequences of wheel-running behaviour in Djungarian hamsters (*Phodopus sungorus*)

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ZUSAMMENFASSUNG

Die vorliegende Arbeit behandelt das nach wie vor ungeklärte Phänomen der Laufradaktivität sowie deren Einfluss auf die Physiologie, Morphologie und Ausprägung biologischer Rhythmen bei Dsungarischen Zwerghamstern (*Phodopus sungorus*). Zwei Eigenschaften machen diese nachtaktive Nagerart aus Zentralasien zu einem sehr interessanten Tiermodell: Neben einer hohen Laufbereitschaft zeigen die Hamster eine ausgeprägte Anpassung an das kontinentale Klima mit extrem kalten Wintern. Gesteuert durch die abnehmende Tageslänge werden im Herbst die Gonaden zurückgebildet und das Körpergewicht reduziert. Zudem wechseln die Tiere vom grau-braunen Sommerfell zum besser isolierenden weißen Winterfell und können außerdem Energie sparen, indem sie die Körpertemperatur für einige Stunden auf minimal 14°C absenken (Torpor). Zur Umkehrung dieser Vorgänge kommt es, wenn die Hamster gegen Ende des Winters, oder nach entsprechend langer Haltung in einem künstlichen Licht-Dunkel-Wechsel mit kurzer Photophase (z.B. LD 8:16), gegenüber dem Kurztag-Signal „refraktär“ werden. Um zu sehen, ob sich die Bedeutung der Photoperiode für die Saisonalität der Hamster (Photoperiodismus) auch im Aktivitätsrhythmus widerspiegelt, wurde der täglich wiederkehrende Wechsel von Aktivitäts- und Ruhephase im Jahresverlauf aufgezeichnet. Es konnte eine durchgehend enge zeitliche Beziehung gezeigt werden zwischen Aktivitätsbeginn und Abenddämmerung sowie zwischen dem Ende der Aktivitätsphase und der Morgendämmerung. Die Länge der Aktivitätsphase war damit im Jahresverlauf eng gekoppelt mit der Dauer der Nacht. Im Herbst und Winter war jedoch der Tagesrhythmus der Hamster ohne Laufrad schwächer ausgeprägt als bei den Tieren mit Laufrad oder fehlte sogar vollständig. Dies bestätigt die Theorie, dass Laufradaktivität einen stabilisierenden Einfluss auf die Rhythmik der Tiere hat. Die Laufradaktivität hatte darüber hinaus starken Einfluss auf die Ausprägung verschiedener Merkmale der saisonalen Anpassung. Der typische saisonale Verlauf des Körpergewichts wurde durch einen Wachstumseffekt verhindert, und die Phase der zurückgebildeten Hoden war verkürzt. Außerdem zeigten die Hamster mit Laufrad keinen Torpor, wohingegen die Fellumfärbung unbeeinflusst blieb. Die Körperzusammensetzung war ebenfalls verändert und wies einen konstant niedrigen Fettanteil auf. Ein weiteres Experiment lieferte überzeugende Hinweise darauf, dass Licht auch bei dieser nachtaktiven Spezies Laufradaktivität unterdrückt (negative Maskierung) und damit die zeitliche Beziehung zwischen dem äußeren Zeitgeber und dem Aktivitätsrhythmus verändert. Dass auch die innere Uhr direkt beeinflusst werden kann, zeigte sich durch eine verlängerte Periodenlänge des freilaufenden Aktivitätsrhythmus nach Veränderung der Außentemperatur unter konstanten Bedingungen (Dauerdunkel). Da sich jedoch nur in einer von zwei Gruppen die Aktivitätsmenge geändert hat, ergeben sich Zweifel an der gängigen Hypothese, dass der Aktivitäts-Level allein für die beobachtete Veränderung der endogenen Periode verantwortlich ist. Insgesamt bestätigen die vorliegenden Ergebnisse die Annahme, es handle sich bei der Laufradaktivität um ein Labor-Artefakt, unterstützen aber ebenfalls die Einschränkung, dass möglicherweise ein natürliches Verhalten lediglich verstärkt oder verändert ist. Einerseits müssen potentielle Effekte bei der Planung zukünftiger Experimente berücksichtigt werden, andrerseits könnten die Einflüsse auf das Körpergewicht, die Hodenentwicklung und Torpor hilfreich sein bei der weiteren Erforschung der Regulation dieser Parameter.

Laufradaktivität • Saisonale Anpassung • Körpergewicht

SUMMARY

The present thesis deals with the still unexplained and baffling phenomenon of wheel-running activity. In Djungarian hamsters (*Phodopus sungorus*) the characteristics of wheel running were examined as well as its effects on physiology, morphology and the occurrence of biological rhythms. This species provides an excellent animal model due to a high motivation to run in a wheel and its evolved seasonal adaptation composed of several traits that change during the year. In anticipation of the harsh winter in the steppes of Central Asia the animals reduce their body weight, change the fur colouration which is combined with improved insulative properties and become reproductively inactive due to regression of their gonads. Finally, daily torpor contributes to saving energy. This shallow form of controlled hypothermia is restricted to the daytime and may last several hours. Winter acclimatisation already begins in late summer and is mainly driven by decreasing day length. At the end of the winter the hamsters become refractory for the ‘inhibitory’ short-day signal and the processes are reversed. In order to see whether dependence on the photoperiod (photoperiodism) is reflected by the daily activity/rest cycle in the course of the year the annual activity pattern was registered. The examination revealed a close temporal relationship between the activity onset and dusk as well as between the cessation of activity and dawn throughout the year, i.e. a strong correlation between the duration of the nightly activity phase and the night length. However, during autumn and winter the daily rhythm was weaker in hamsters without a wheel compared to exercising animals, or it even disappeared. This finding is well in accordance with the hypothesis that wheel running has a stabilising effect on rhythmicity in this species. In addition, wheel-running activity strongly affected different traits of the seasonal acclimatisation. For instance, the typical seasonal body weight cycle was prevented due to a growth-stimulating effect of running exercise, and the phase of involuted testes was shortened. Moreover, daily torpor was completely inhibited in hamsters with running wheel access, whereas the fur colour change was unaltered. The body composition of exercising hamsters was also affected and reflected a constant low body fat content. A further experiment provided evidence for a suppressing effect of light (negative masking) on wheel running which may cause alterations in the phase relationship between the exogenous time cue and the overt behavioural rhythm. A direct influence on the internal clock was demonstrated by means of the free-running activity rhythm under constant darkness with changes in the ambient temperature (T_a). Indeed, two groups of hamsters exhibited an altered length of the free-running period subsequent to the change in T_a. However, only in one group the amount of wheel-running activity was changed which contradicts the notion that such alterations in the period length are linked to changes in the activity level. In conclusion, the present results argue for the assumption that wheel running is a laboratory artefact but, equally, support the possibility of an artificially enhanced or altered natural behaviour. On the one hand, the findings point to the importance of considering potential effects when wheel-running activity is involved in prospective experiments. On the other hand, the impact of running exercise on body weight, testis cycle and torpor as shown in this thesis might be helpful for investigations of the regulation of these parameters.

Wheel-running activity • Seasonal adaptation • Body weight

CONTENTS

1

Introduction

Movement is one of the fundamental properties of animals. The importance of motility is reflected by the variety of adaptive locomotion ranging from swimming to flying. Almost all areas of the animals' life are directly dependent on mobility. For example, for sexual reproduction a mating partner that in general itself is moving about has to be found. In this regard, locomotion is essential for the continuity of the species. Survival of individuals and successful reproduction in turn is intimately connected with sufficient food supply which is impressively reflected by annual migrations predominantly in mammals and birds. Populations of the Wildebeest (*Connochaetes taurinus*) migrate up to 3,000 kilometres per year, and the Arctic tern (*Sterna paradisaea*) even covers the ten fold distance each year during the flight from the Arctic breeding grounds to the Antarctic and back. Although these examples represent extremes they nevertheless demonstrate the importance of mobility. In this context, it appears to be necessary to mention that locomotion in animals usually has no end in itself but is purposive with respect to the basic needs such as feeding. But due to the associated increase in energy consumption a kind of cost-benefit calculation has to underlie the animals' activity behaviour. Modern day humans, in contrast, use exercise to improve their well-being, which, from a physiological point of view, is an energy-wasting behaviour. However, this loss of stored energy, preferentially fat, is only feasible at times of opulence and is highly desired in many cases of human voluntary exercise. Long-term benefits on physical and mental health are probably the major motivations for frequent physical exertions that have no obvious immediate goals. The latter fact also applies to voluntary wheel running which comprises a significant portion of activity in many species including rodents. Therefore, the question arose whether the animals' wheel-running activity exhibits parallels to human exercise (Eikelboom 1998). It is worth mentioning that considerable endurance performance in men may lead to elation, the so-called runner's high, which is due to a massive release of endorphins and, thereby, may even result in an exercise addiction. Such a consequence in animals caused by wheel running is very unlikely for several reasons. Rodents that obtain access to a running wheel usually show a high level of exercise right from the start, indicating the wheel per se to be highly attractive for the animals. Indeed, it is also normal that the amount of wheel-running activity increases within the first days or weeks giving the impression of an increasing dosage. The explanation for this rise, however, is rather less spectacular but convincing. It is assumed that instead of showing ‘drug abusing behaviour’ the animals simply need some practice (in a wheel) before they show a stable pattern of usage (Sherwin 1998b). According to this assumption, wheel-running activity usually decreases after a certain time of free access, indicating that the motivation to run gradually weakens over time (Gattermann et al. 2004).

With respect to a biological perspective, wheel running initially was tried to be explained by equivalent natural behaviours rather than artificially evoked motivation. Accordingly, running exercise was suggested to be a substitute for searching or exploratory activity (for review, see Mather 1981), presumably also due to difficulties in assuming that an animal’s behaviour might be performed for its own sake. However, the substitution theory as exclusive explanation is strongly contradicted by studies where rodents showed pronounced wheel running regardless of whether the environment was massively enriched or not (Brant and Kavanu 1964, 1965, Sherwin 1998a). The results point to the high motivation of animals to run in a wheel, which is proven by several intriguing findings. For instance, in rats the food intake was depressed for several days when provided with a running wheel (Looy and Eikelboom 1989). If, however, feeding was linked to the possibility of running in a wheel, rats increased eating to gain wheel access (Timberlake and Wozny 1979). The importance of wheel running for animals was also demonstrated with operant tasks or aversive environments where the strength of motivation was tested by means of unlocking a wheel (Belke and Heyman 1994, Collier et al. 1990, Iversen 1993), or gaining access to areas containing a wheel (Sherwin 1996, 1998a, Sherwin and Nicol 1996). Sherwin (1998b) met the challenge of finding a general interpretation for this distinct behaviour by considering a tremendous number of studies on internal and external causal factors as well as potential functions of wheel running. He came to the plausible conclusion that ‘wheel running has no directly analogous naturally occurring behaviour’, is ‘self-reinforcing’ and ‘may be an artefact of captive environments or of the running wheel itself’.

Despite the unknown ‘nature’ of the behaviour, several decades ago wheel-running activity evolved as an important tool in a certain field of biology, namely chronobiology.

Measurements of the periodically occurring processes are naturally indispensable for investigations of biological rhythms. In this regard, for determination of for example, the activity/rest cycle, wheel running provides a parameter that is easy (and non-invasive) to measure. In contrast to the general activity of animals, records of running exercise usually contain considerably less noise, and therefore onsets and offsets within the daily activity pattern are easier to determine; and exactly those reference points are the basis for precise rhythm analyses. Furthermore, registration of wheel revolutions enables an accurate quantification of the corresponding activity behaviour which becomes important when for example, external factors such as light or ambient temperature are varied to reveal their influence on the activity pattern. In general, the overt activity/rest cycle of animals is seen as a behavioural output that is driven by the internal clock. The relationship between the daily occurrence of activity/rest and the endogenous pacemaker, which is localised in the hypothalamus (suprachiasmatic nuclei, SCN) of mammals (Moore and Eichler 1972, Stephan and Zucker 1972), becomes apparent when external time cues are excluded. Under such conditions with a constant ambient temperature and without a light-dark cycle, i.e. either constant light (LL) or constant darkness (DD), an activity rhythm is still present with a period usually slightly deviating from 24 hours (circadian). This free-running period (τ) impressively reflects the functionality of the inherent clock. In contrast to a constant environment, under natural conditions the circadian rhythm of the endogenous clock is entrained by the 24-hour night-dark cycle that is the most potent ‘zeitgeber’ (external time cue) for daily biological rhythms. However, light does not only adjust the period and/or phase of the internal oscillations in the SCN but also directly effects the overt output rhythm, i.e. activity. In nocturnal rodents, e.g., activity is suppressed by light which is called negative masking (for review, see Mrosovsky 1999). Hence, under conditions with a light-dark cycle (24 h) the observable activity rhythm of an animal results from the influences of both the internal clock that is entrained by the light-dark alternation and the light-dark cycle itself. One component is still lacking in this general model of the mechanisms showing how the period of the overt activity rhythm is generated, namely the activity itself. Considerable amounts of activity, e.g. wheel running during the light phase of a light-dark cycle can pronouncedly shift the phase of the activity rhythm by several hours in nocturnal Syrian hamsters (*Mesocricetus auratus*) for instance, which was observable when the hamsters were released into DD subsequent to the day of access to a novel wheel in the rest phase (Janik and Mrosovsky 1992, Mrosovsky and Biello 1994).

Under constant conditions those phase shifts might also be connected to a change in the free-running period (Weisgerber et al. 1997). Although some correlations between the occurrence of both effects and the number of revolutions were found, the findings of increased wheel running due to a lowered ambient temperature without enhanced effects indicated that a particular amount of activity on its own is not sufficient but additionally needs a high motivation (Janik and Mrosovsky 1992, Mrosovsky and Biello 1994). This is in accordance with an early proposition of Aschoff (1960) who suggested that the ‘level of excitement’ is involved in generating the period length of activity rhythms. In following investigations this term was equated with the activity level, or more concretely, with the amount of activity which appeared to be too simplified (Koteja et al. 2003). Nevertheless, there is no doubt as to the feedback effects of pronounced exercise on the internal clock. On the one hand, this fact reflects the complexity of how an observed period length and phase is generated, and on the other hand, it becomes apparent that the extensive knowledge about the influences of wheel running is essential for the further use of this parameter in biological rhythms research.

Apart from effects on the occurrence of rhythms, running exercise was shown to evoke various impacts in rodents. For instance, wheel running enhances hippocampal neurogenesis and learning in mice (Van Praag et al. 1999). Furthermore, several physiological and morphological effects have been demonstrated in Syrian hamsters that display seasonal changes in gonads, body weight and incidence of deep hibernation. Short photoperiod-induced gonadal regression is incomplete in hamsters with access to a running wheel (Gibbs and Petterborg 1986), although the neural integration of the photoperiodic signal is not prevented (Menet et al. 2005). Hibernation is even completely inhibited by wheel running in this species (Menet et al. 2003). The most striking finding was the exercise-induced increase in body mass (Borer and Kaplan 1977, Borer and Kooi 1975, Gattermann et al. 2004) which is caused by growth (Borer and Kelch 1978, Borer and Kuhns 1977).

There is also evidence of wheel running interfering with seasonal acclimatisation in Djungarian hamsters (*Phodopus sugorus*, also known as Siberian hamster) insofar as the short day-induced body mass reduction was affected as well as the frequency of shallow daily torpor (Thomas et al. 1993). The marked seasonality of these dwarf hamsters reflects the strong annual changes of their natural environment. The species inhabits the steppes of eastern Kazakhstan, western Siberia and the Kraznoyarsk region of Russia along the

Yenissei River (Ross 1998). This information, however, is based on relatively old data and therefore is possibly obsolete. Nonetheless, the distribution area is characterised by a continental climate with extremely cold winters. Thanks to an evolved seasonal adaptation in several physiological and morphological traits the small rodents are able to cope with the harsh environmental conditions in winter. The change from the summer hamster to the winter phenotype is driven by decreasing day length via the correlating length of the nightly secretion of pineal melatonin (Bartness and Goldman 1988, Hoffmann 1979, Hoffmann et al. 1986, Steinlechner 1992). As already mentioned above, the animals reduce their body mass during short-day acclimatisation (Hoffmann 1973, Steinlechner et al. 1983) instead of increasing fat stores which is only reasonable for larger animals. Due to the decreased body mass the total energy consumption is lowered although the increasing surface to volume ratio facilitates heat loss (Heldmaier and Steinlechner 1981b). This disadvantage, however, is overcompensated by the more insulative properties of the white winter fur compared to the greyish-brown summer fur. Daily torpor is another winter trait that helps to save energy (Heldmaier and Steinlechner 1981a). The temporal occurrence of torpor events is under the control of the endogenous timekeeping system and restricted to the rest phase of the animals, i.e. in the daytime (Ruf et al. 1987). Accordingly, single torpor bouts may last up to eight hours. Generally, body temperature does not drop below 14°C during this shallow form of controlled hypothermia compared to deep hibernation (Heldmaier et al. 1989). Increased thermogenic capacity of brown adipose tissue (BAT) in short days is not only helpful for re-heating the body of torpid hamsters but is the fundamental adaptation for coping with severe cold (Heldmaier et al. 1982). For this reason, it is intriguing that the seasonal body mass decline is mainly due to a reduction in fat mass (Wade and Bartness 1984, Klingenspor et al. 2000) including BAT (Rafael et al. 1985), pointing to the selective advantage of a lowered body mass in winter. This short day-induced reduction in body mass was shown to be precisely regulated (Steinlechner et al. 1983) suggesting a ‘sliding set-point’ (introduced by Mrosovsky and Fisher 1970). After a temporary food deprivation during the course of body mass reduction, hamsters regained weight when again provided with abundant food amounts matching those values of the control animals that showed a regular decline in body mass. However, the underlying regulation mechanism of the seasonal body weight cycle that is linked to seasonal adiposity has not yet been fully understood. The initial voluntarily decreased food intake, as well as the depletion of stored fat implicates brain regions that are involved in regulation of energy balance such as the hypothalamic arcuate nucleus (ARC). Accordingly, photoperiodic influences on gene expression were shown for several factors that are involved in the acute or homeostatic control of energy balance, e.g. proopiomelanocortin (POMC), agouti-related protein (AGRP), cocaine- and amphetamine-regulated transcript (CART) and the leptin receptor (OB-Rb), but not for neuropeptide Y (NPY) known for its orexigenic effect (Mercer et al. 2000, 2003). Furthermore, the regulation of histamine H3 receptor (H3R) was demonstrated to play a potential role in seasonal physiology of Djungarian hamsters (Barrett et al. 2005) as has been shown together with histamine synthesis in the annual cycle of hibernation of the ground squirrel (*Citellus lateralis*, Sallmen et al. 1999, 2003a-c). The stomach-derived hormone ghrelin is regarded to have opposing (i.e., stimulating) effects on appetite and body mass in contrast to leptin but does not seem to play a crucial role in modulating the long-term seasonal weight cycle (Tups et al. 2004). In contrast, hypothalamic responses to circulating leptin, which is produced by adipocytes, appear to be important for the reduction in body mass as well as for recovery. It was shown that the fat long-day hamsters with already high blood leptin concentrations are less sensitive to exogenous leptin than the lean short-day individuals with an appropriate low leptin level (Klingenspor et al. 2000, Rousseau et al. 2002). This seasonal change in leptin sensitivity is supposed to be the explanation for the paradoxical situation of a decreasing leptin level due to reduced body fat which does not interfere with a further body weight decline during short-day acclimatisation and vice versa when the hamsters have become photorefractory (Rousseau et al. 2002). More recent studies have revealed that the suppressor of cytokine signalling 3 (SOCS3) is involved in the regulation of seasonally changing leptin sensitivity in Djungarian hamsters (Tups et al. 2004, 2006) and field voles (*Microtus agrestis*; Król et al. 2007). However, it is still unclear whether the explained mechanism only provides the prerequisite for a seasonal change in body mass by switching from high leptin sensitivity to resistance (or vice versa) or even enables the precise regulation of a seasonally appropriate weight. Therefore, elucidation of the mechanisms by which ‘defended body weight’ is adjusted remains a major research objective (Mercer et al. 2003). Although the regulation of, for example, daily torpor and the seasonal weight cycle still has to be investigated, the seasonal traits of Djungarian hamsters are well characterised. Thus, the species provides an excellent animal model for investigations of wheel-running effects on physiology and morphology also with respect to seasonality. Moreover, the Djungarian hamster is a widely-used species in biological rhythms research which substantiates the need for information about potential impacts of wheel-running activity not only on physiology but also on rhythmicity. Therefore, the aim of the present thesis was to provide further knowledge about a distinct behaviour that is used as hand of the clock, but still is a conundrum. For investigating the characteristics and consequences of wheel running in this species different experiments were carried out. In the first part of the study (chapter 2) the ambient temperature was varied in combination with two lighting conditions to examine the influence of ambient temperature on the amount of wheel running in a light-dark cycle and on the free-running period in constant darkness. The second part focussed on the daily activity pattern in the course of the year (chapter 3). Since the seasonal acclimatisation is triggered either by decreasing photoperiod incipient in late summer or by becoming refractory for the ‘inhibiting’ signal of short photoperiods in late winter, exposure to natural lighting conditions was chosen to see whether the hamsters’ photoperiodism is reflected by the photoperiodic entrainment of the annual activity pattern. In the last part of the study (chapters 4 and 5), influences of wheel running on physiology, morphology and behaviour were examined with particular attention paid to the most striking property in this species, i.e. seasonality.

2

Effects of changes in ambient temperature on wheel-running activity and the free-running period

ABSTRACT

Exposure of female Djungarian hamsters (*Phodopus sungorus*) to different ambient temperatures (T_as) revealed a significantly decreased amount of wheel-running activity at a thermoneutral T_a (26-27°C) compared to a common housing temperature (22.5°C). However, lowering of the T_a to 15°C did not evoke a further increase of wheel running. The results of repeated exposure to 22.5°C showed a considerable decrease of total daily revolutions (~30%) within ten weeks. Comparison to a second group differing in the age of the animals at their initial RW access indicated that the decline in the amount of running was related to the duration of RW access rather than an age effect. Analysis of the phase relationship between the onset and offset of wheel running and the zeitgeber revealed no significant changes in the entrainment of the activity rhythm to the light-dark cycle of 16 h of light and 8 h of darkness. Under constant darkness, however, the free-running period (τ) was significantly altered after a change of the T_a. The direction of the change in period length was similar for two groups of hamsters, irrespective of whether the initial T_a (22.5°C) was lowered (15°C) or elevated (26°C). In the case of increased T_a wheel-running activity significantly decreased, so that the lengthened τ was well in accordance with the assumption of a negative correlation between the activity level and the period length of the circadian rhythm. Contradicting, the animals of the second group maintained the number of revolutions subsequent to the decrease in T_a but even significantly increased τ compared to the first group, indicating influences on τ independent of the activity level.

INTRODUCTION

Wheel-running activity is commonly known to influence the daily body temperature (T_b) rhythm in rodents. In Syrian hamsters (*Mesocricetus auratus*), for instance, not only the amplitude is increased in exercising animals compared to sedentary controls (Golombek et al. 1993) but after three weeks of running wheel (RW) access also the T_b level during the light phase is elevated while an acute effect due to running is lacking (Conn et al. 1990). The brain temperature, however, even decreases in the same species immediately after the start of running activity and recovers rapidly subsequent to its cessation (Gordon et al. 1981), demonstrating one aspect of thermoregulation contributing to the pattern of the body temperature rhythm. Consistently, chronic thermal stimulation of the anterior hypothalamus greatly inhibits free-running activity (Gordon et al. 1980). In this regard it is not surprising that running activity of rodents is influenced by ambient temperature (T_a), mostly characterised by an inverse correlation between locomotor activity and environmental temperature (Clarkson and Ferguson 1972, Fregley 1956, Kauffman et al. 2003).

To investigate T_a effects on wheel running in Djungarian hamsters (*Phodopus sungorus*) that are known to make pronounced use of RWs two groups of animals were exposed to three different T_as with an unequal sequence. Usually, Djungarian hamsters are housed under long-day conditions with a light-dark (LD) cycle of 16 h of light and 8 h of darkness (LD 16:8) at a T_a of 20-23°C. For those long day-acclimatised animals, unlike the winter phenotype, even 23°C is below the thermoneutral zone with a lower critical value of ~26°C (Heldmaier and Steinlechner 1981b). Therefore, in addition to a common housing temperature (22.5°C) which is equivalent to moderate cold (cool) for the hamsters, a thermoneutral T_a (26-27°C) was chosen as well as a cold T_a (15°C). Furthermore, the study was extended by subsequent exposure to constant darkness (DD) combined with an alternation in T_a after several weeks. Those constant conditions except for the unique change in T_a were required in order to determine how the level of locomotor activity influences the period of the free-running activity rhythm. A couple of laboratories has already tried to answer this question by using several species. Some findings were well in accordance with the proposition of Aschoff (1960) that the length of the free-running period is negatively correlated with the ‘level of excitement’ (Edgar et al. 1991, Mistlberger and Holmes 2000, Yamada et al. 1988), but others were not (Aschoff et al. 1973, Deboer and Tobler 2000). Conflicting results were even found for the same species (*Mesocricetus auratus*) in the same laboratory (Mrosovsky 1999). The present study on Djungarian hamsters should provide further data for the ongoing discussion about the relation between the activity level and the free-running period.

MATERIALS AND METHODS

Djungarian hamsters (*Phodopus sungorus*) were bred and raised outdoors under natural lighting conditions (~52° N latitude) with natural ambient temperatures. After weaning the animals were housed seperately in polycarbonate cages (Makrolon type II) with food (hamster breeding diet, Altromin 7014) and tap water available ad libitum, supplemented by a slice of apple once a week.

On June 16th (2004) female hamsters (~2 months old) were transferred into two temperature-controlled chambers (22.5 ± 1°C, each) with a light-dark cycle of 16 h of light and 8 h of darkness (lights on from 5:00 h to 21:00 h; CET). Ambient temperature (T_a) was measured and stored with temperature loggers (DS1921L: range −20-85°C; resolution 0.5°C; iButton, Maxim Integrated Products, Inc., Sunnyvale, CA). With the beginning of the experiment the animals obtained access to a running wheel (~14 cm inner diameter) in their home cages. Wheel turns were registered with the help of a magnet fixed to each wheel and a reed switch on every cage lid. Signals were continuously conveyed to a personal computer, summed up and stored in 6-min-intervals. After the initial phase at the common housing T_a of ~22.5°C the animals were sequentially exposed to different T_as (~15°C, ~27°C) and, at the end of the experiment, also in combination with constant darkness (DD; dim red light < 1 lx). The sequence of varied T_as was different in the chambers; for respective sequence and duration of T_a phases see Fig. 2.1. During the experiment some animals had to be substituted due to death or because of avoiding the wheel; one individual became blind. Despite substitution the sample size was reduced for comparisons of paired samples (repeated measures).

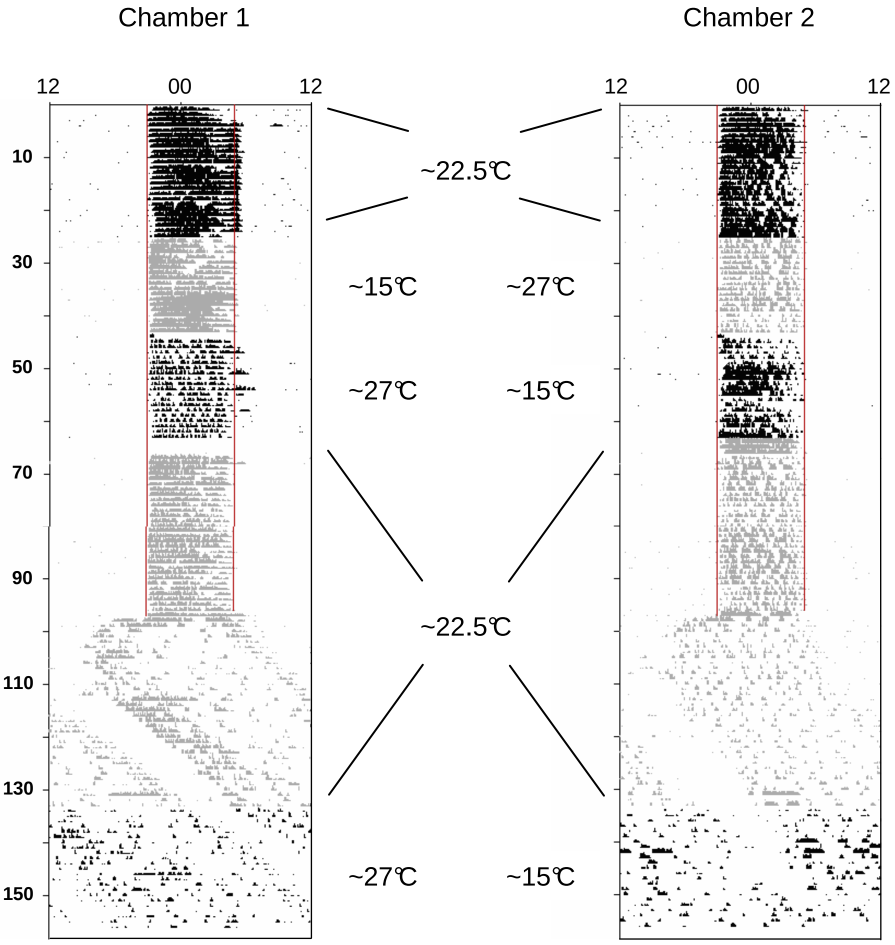

Figure 2.1. The experimental design is depicted by activity records (actograms; single plots) of two individuals. Consecutive days (from 12:00 h to 12:00 h) are plotted one below the other. The squence of the varied ambient temperature (T_a) was different for both groups. Durations of exposure (days on left side) to specific T_a are identifiable/denoted by different grey values for activity illustration. The lighting conditions are indicated either by vertical lines for lights-off and lights-on (LD 16:8) ore by the lack of those lines (DD).

For statistical analyses of wheel running ten days out of experimental phases 1-4, respectively, were averaged for each animal. Identical consecutive days were chosen for each individual from the end of the phases, if not impossible due to failures in data acquisition or obviously unrepresentative low daily amounts of wheel running. Activity onsets and offsets were determined by comparison of raw data and an activity threshold resulting from the same raw data set but processed with a moving average of 24 hours and finally lowered by 50% (for details, see chapter 2). Period calculation of the free-running rhythms were carried out with the software Peanuts written by Thomas Ruf (Lomb-Scargle periodogram; Ruf 1999, Van Dongen et al. 1999); from the data of DD conditions at least the first 12 days were excluded due to individual decompression of the activity phase obscuring the rhythm. Determinations of the period (with ~20 consecutive days; at least 12) and the amount of wheel running (for all days in DD except for the first 12) were carried out for the two phases in DD with different T_a (Fig. 2.1). Differences were considered statistically significant when $P < 0.05$. Values are given as means and SEM.

RESULTS

In the first part of the study two groups of hamsters were exposed to three different ambient temperatures (T_a) within four consecutive experimental sections (Fig. 2.2). The initial and final T_a phase (~22.5°C; cool) were similar for both groups unlike the sequence of the thermoneutral (~27°C) and cold phase (~15°C) in-between. Regarding both cool phases comparison of the amount of wheel-running activity revealed no group difference, respectively (Fig. 2.3). Within each group, however, exercise during the final phase was significantly decreased compared to the initial phase with the same T_a. In the first group wheel-running activity at ~27°C (following ~15°C) was significantly reduced compared to all remainig phases. In contrast, in the second group wheel-running activity at the thermoneutral T_a was only reduced compared to the previous cool phase, and significantly higher in comparison to the thermoneutral phase of group 1.

Some hamsters obtained access to a RW only with the beginning of the fourth experimental phase (~22.5°C). During this phase they produced a significantly higher number of revolutions than the remaining hamsters of the same age which were provided with a wheel several weeks earlier (Fig. 2.4). If, however, wheel-running activity was not considered/compared with respect to the age of the animals but with view to the elapsed time of free RW access, i.e. the wheel running ‘history’, a difference was not found anymore.

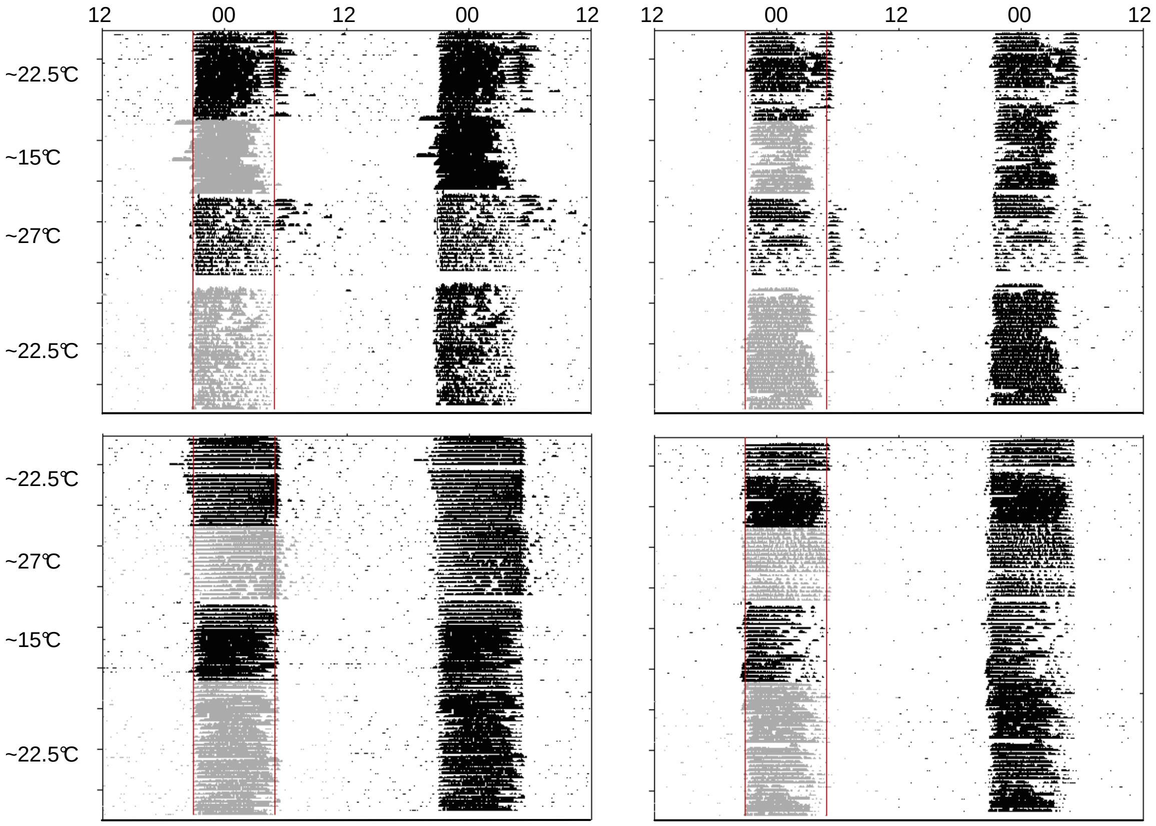

Figure 2.2. Actograms of four individuals exposed to varied T_a in LD 16:8. The sequence of T_a phases was different (~27°C and ~15°C) for/between two groups (upper graphs = chamber 1; lower graphs = chamber 2). Successive days are depicted as douple plot, i.e. in rows (48 h) as well as one below the other. Vertical lines indicate lights-off and lights-on. Empty rows are due to failures in data acquisition.

Analyses of the phase-angle differences between activity onset and lights-off as well as between activity offset and lights-on were carried out to clarify, whether entrainment of activity was influenced by varied T_a. Within both groups there was no significant change neither in timing of the onset nor in temporal occurrence of the offset with respect to the different T_as (Fig. 2.5). However, activity offsets were delayed in the thermoneutral phase (~27°C) of group 2 when compared to the cold (~15°C) and (second) cool phase (~22.5°C) of group 1.

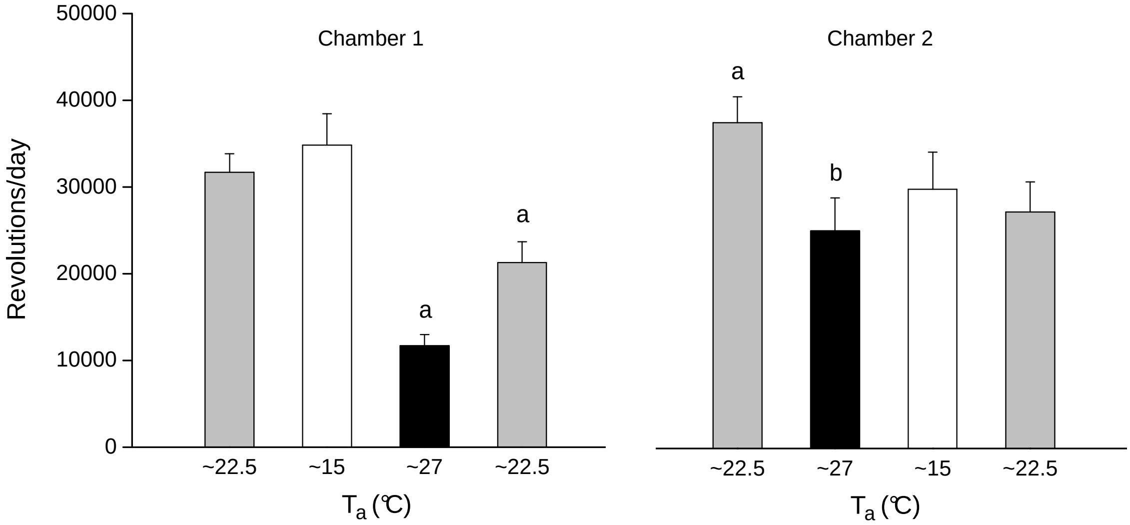

Figure 2.3. Amount of wheel-running activity (mean + SEM) at different ambient temperatures (T_a). The sequence of changes in T_a (from left to right) was different for both groups (1, $n = 7$; 2, $n = 5$). Friedman RM ANOVA on ranks revealed differences within group 1 ($P < 0.01$) and group 2 ($P < 0.05$) further analysed by pairwise comparison (Student-Newman-Keuls). Phases of the same T_a were compared between groups with Mann-Whitney rank sum test; a = significantly different from all other experimental phases within the group ($P < 0.05$); b = significantly different from the corresponding T_a phase (~27°C) of group 1 ($P < 0.05$).

After about five weeks of DD the T_a (~22.5°C) was either elevated to ~26°C (chamber 1) or reduced to ~15°C (chamber 2), as shown in Fig. 2.6. Both the increase as well as the decrease of T_a led to a significantly elongated free-running period ($P < 0.05$; Wilcoxon matched pairs test; Fig. 2.7). However, the mean amount of period elongation (h) was significantly higher in the latter group that was exposed to cold (0.50 ± 0.23 vs. 0.16 ± 0.06; $P < 0.05$; Mann-Whithney rank sum test). Despite this finding there was no difference in the absolute τ values between the groups, neither before nor after the T_a change.

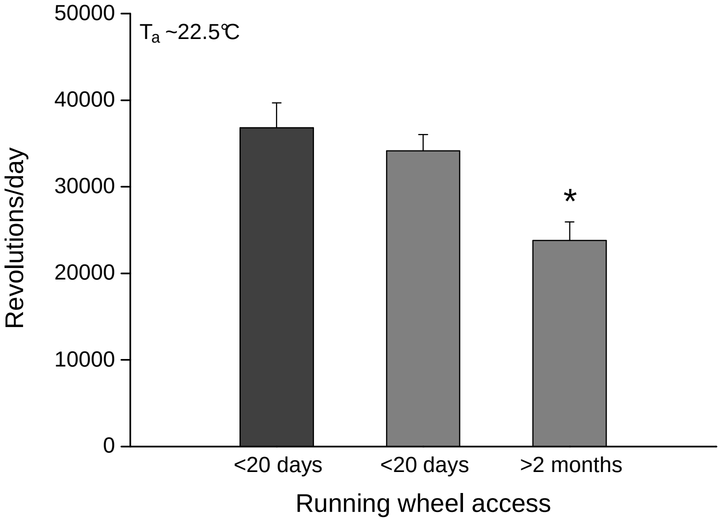

Figure 2.4. Wheel-running activity (mean + SEM) of hamsters with either more than two months of free access to a running wheel (RW) or only less than 20 days prior to the ten analysed days. The first and third column represent different groups of hamsters ($n = 6$; $n = 12$) with the same age (~5 months old) but different with respect to the duration of the proceding RW access. The second column stands for the same individuals as column 3, but the animals were younger (~10 weeks old), and the RW 'history' was similar compared to the animals represented in column 1. The Kruskal-Wallis ANOVA on ranks revealed significant differences ($P < 0.01$) differentiated by Dunn's post-hoc test; * significantly different ($P < 0.05$) from remaining data sets.

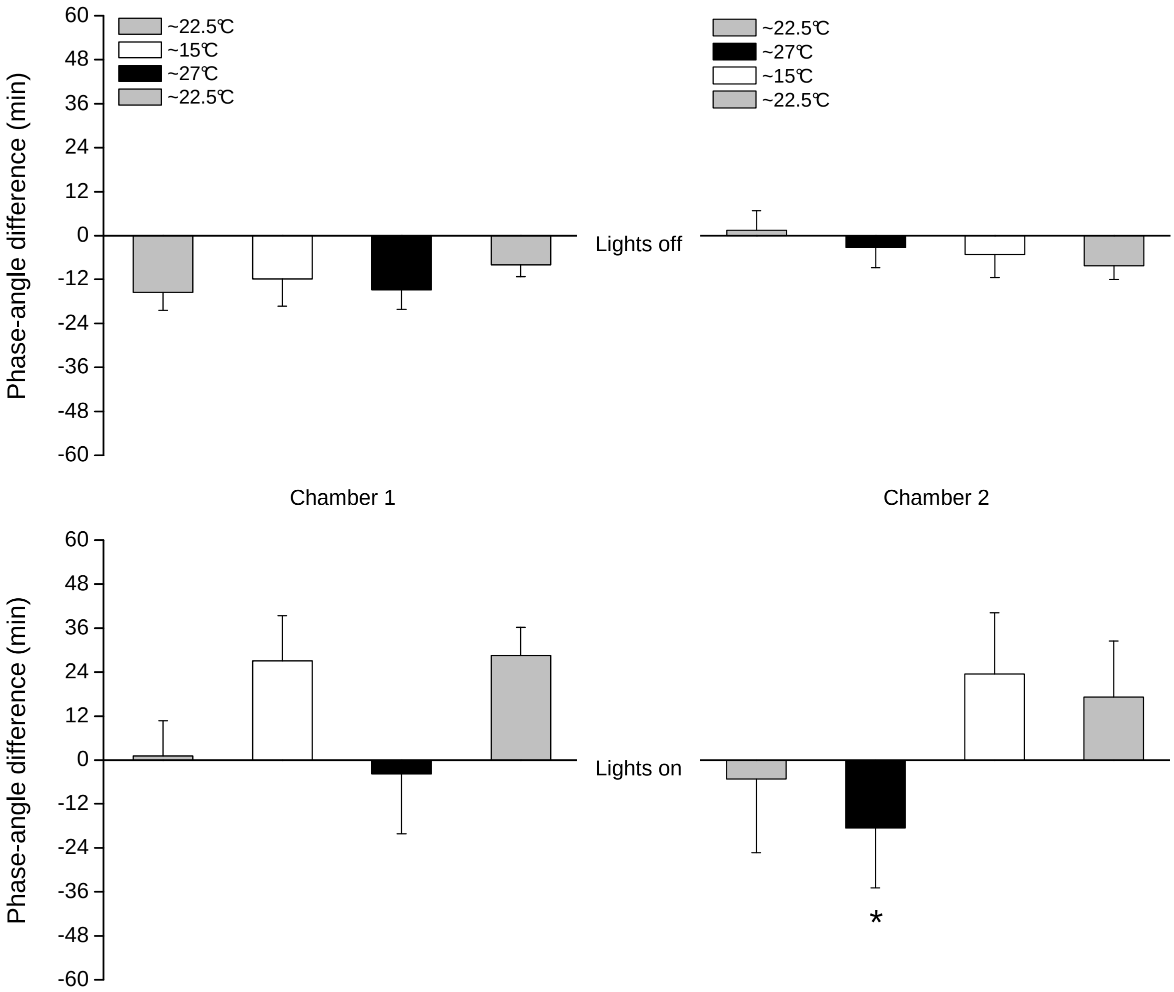

Figure 2.5. Temporal relation of mean (and SEM) activity onsets and offsets to the corresponding phase-reference point of the zeitgeber (lights off, lights on), respectively, at different T_a; * significantly different from the second (~15°C) and fourth experimental phase (~22.5°C) of the first group ($P < 0.05$; Mann-Whithney rank sum test).

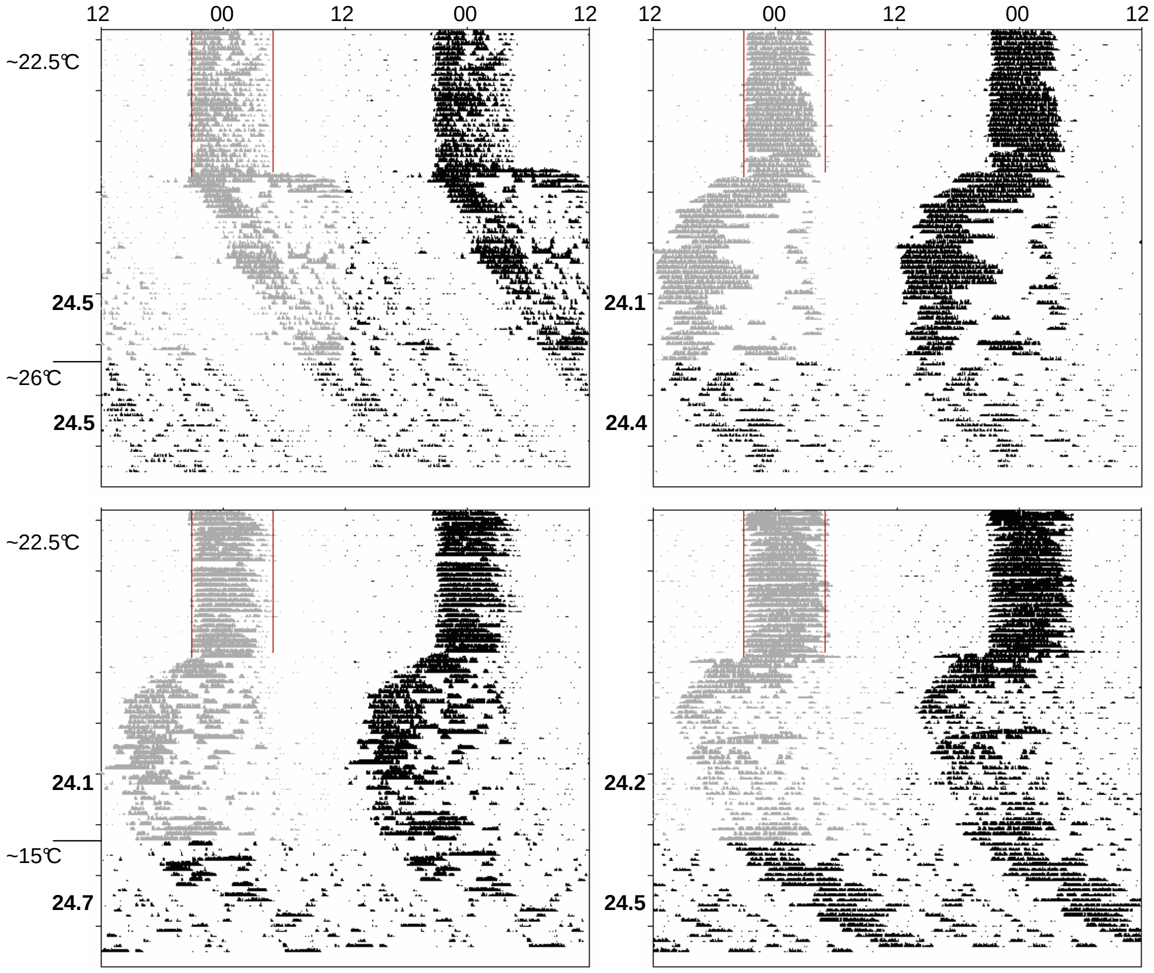

Figure 2.6. Actograms (double plots) of four individuals that were released into constant darkness (DD) after exposure to LD 16:8. During exposure to DD T_a (initially ~22.5°C) was either increased up to ~26°C (upper graphs; chamber 1) or lowered (down) to ~15°C (chamber 2). The respective free-running period (h) of either before or subsequent to the T_a change in DD is shown.

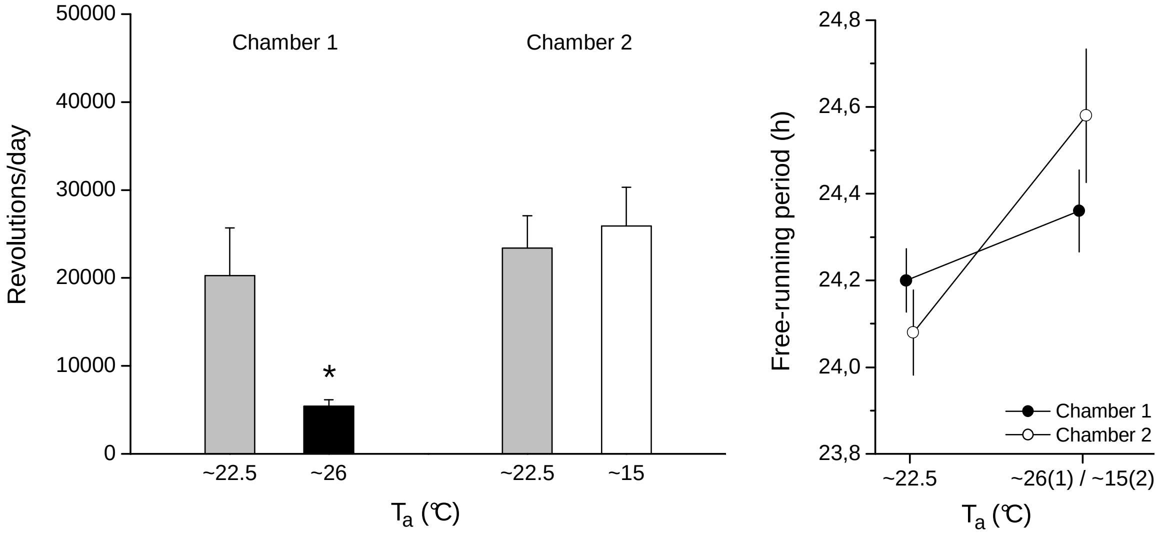

Figure 2.7. Wheel-running activity (left) and free-running periods (right) of two different groups ($n = 9$; $n = 8$) before and after a change in T_a under constant darkness. For the amount of revolutions per day Kruskal-Wallis ANOVA on ranks revealed significant differences ($P < 0.001$) differentiated by Dunn's post-hoc test; * significantly different ($P < 0.05$) from remaining data sets. The free-running period of both groups was significantly increased after the change in T_a ($P < 0.05$; Wilcoxon matched pairs test). This increase was more pronounced in the second group ($P < 0.05$; Mann-Whithney rank sum test).

DISCUSSION

The present results indicate an exercise-suppressing effect of thermoneutral T_a in comparison to temperatures beneath the lower critical temperature (T_{lc}) of ~26°C for long day-acclimatised hamsters (Heldmaier and Steinlechner 1981b). Indeed, the animals of the second group were running significantly less during exposure to the thermoneutral T_a only when compared to the previous cool phase but not in comparison to the following cold phase, in contrast to group 1 with a total daily amount of revolutions significantly lower at ~27°C compared to all remaining phases. Inconsistent results, however, were conceivably due to the unequal sequence of differing T_as. Based on this assumption not only the absolute environmental temperature plays a role but also the magnitude of the change and, thus the T_a 'history'. In order to avoid cold acclimation the length of the experimental phase was bounded to the minimum required for a meaningful data analysis. At least visual inspection of the activity records did not reveal a trend in the amount of running during the course of cold exposure. Despite the significantly higher activity of the second group at ~22.5°C (phase 1) compared to ~15°C (phase 3), which might be explained by the different duration of RW access before (see below), alltogether the findings indicate a stimulating influence of ~15°C compared to thermoneutrality rather than an inhibiting effect on the amount of wheel running. In this regard, both T_as below the T_{lc} seemed to be of comparable influence on wheel-running activity. For red squirrels (*Tamiasciurus hudsonicus*) a significant negative correlation between the mean daily running activity and environmental temperature was demonstrated for a wide range reaching from 10 to 35°C (Clarkson and Ferguson 1972).

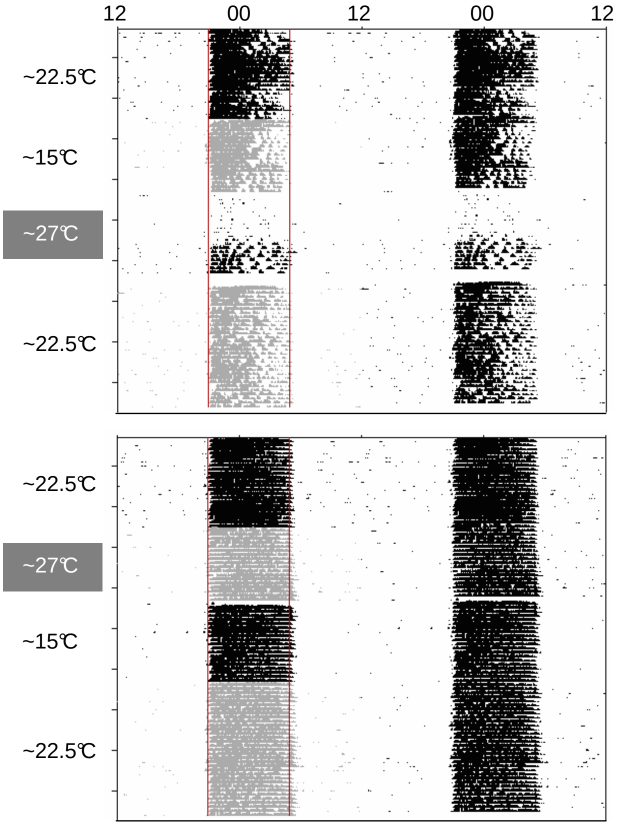

Figure 2.8. Actograms (double plots) of two individuals with extremely different responses in wheel running while exposed to ~27°C. One hamster temporarily avoided the wheel after the increase in T_a, whereas the other animal seemed to be completely ‘unimpressed’ by changes in T_a.

The authors hypothesised that heat generated by exercise may result in hyperthermia and thus, the amount of activity is limited by the level of hyperthermia that is tolerated. It appears unlikely that in the present study the reduction of wheel-running activity at the thermoneutral T_a was due to severe overheating of the animals. Instead, the range of responses reaching from a complete avoidance of the wheel to high numbers of wheel turns only marginally decreased suggests an influence on the individual motivation/urge to run rather than an essential self-protection behaviour (Fig. 2.8).

Albeit the statistical analysis did not reveal any change in the entrainment of activity rhythms due to T_a variations within the groups, there was an apparent tendency similar for both groups. The results for the activity offset indicate that the cessation of wheel-running activity related to lights-on was advanced during the cold phase, and delayed at a T_a of ~27°C. Indeed, the mean offset was slightly advanced during the second cool phase compared to the first one, but this different trend (n.s.) despite the same T_a was similar for both groups and, therefore, rather linked to the number of wheel revolutions per day than reflecting only a pronounced variation of this parameter. Since wheel running significantly decreased within ~10 weeks (~30%), i.e. between the initial phase (~22.5°C) with RW access and the fourth experimental phase with the same T_a, a relation between the daily amount of wheel running and the end of the activity phase (α) is suggested according to the previous assumption. However, relatively high amounts of activity during the cold phase (~15°C) coincide with a tendency of a shortened α due to an advanced offset, implicating an elevated activity level. Furthermore, the results of the thermoneutral phase (~27°C) indicate a tendency of α decompression with delayed offsets. This expansion of wheel-running activity beyond the dark phase is per se very untypical (see chapter 3 and 5) but appeared in several animals during the initial phase of RW access and during the thermoneutral phase. From a behavioural point of view it might be explained as follows: There is no doubt about a strong urge to run that is most pronounced within the first weeks of free RW access (see chapter 3 and 5). Accordingly, it is conceivable that the presumed negative masking effect of light on wheel running was not strong enough to suppress this initial marked motivation to run directly after lights-on. At first view this cannot explain that hamsters overrode the masking effect also during the thermoneutral phase when the activity level was lowest, thus indicating a weak urge to run. However, the explanation is also based on high motivation. Indeed, wheel running was reduced due to the warm environment, but exactly this suppression during the dark phase might have been the reason for a last activity bout subsequent to the zeitgeber's signal for the end of the 'running time'. According to this, both cases demonstrate the conflict between exercise suppression by light and the inherent urge to run in a wheel.

Together, a simple causal relation between the daily amount of wheel-running activity and the length of α appears unlikely. Again, it has to be emphasised that interpretations above are based more on tendencies and individual behavioural responses than on significant findings. In any case, the timing of the activity onset definitely showed no change in response to different T_as under LD 16:8. It appears that individual changes of α due to the timing of activity cessation represent the range of individual wheel-running behaviour rather than clock-controlled changes. Indeed, stability of the activity onset strongly suggests an activity rhythm consistently entrained by the zeitgeber throughout the experimental phases with LD cycle, particularly due to the supposed dominant contribution of the entrained clock to the temporal occurrence of the activity onset (Daan and Aschoff 1975). However, this fact has to be considered carefully, since the close phase relationship might also be a result of negative masking due to light. This light-induced activity suppression has been previously shown for Syrian hamsters (*Mesocricetus auratus*; Redlin and Mrosovsky 1999). On the other hand such a masking effect of light on wheel running would not prevent a more delayed activity onset as found. Together, the onsets almost coinciding with the time when lights were switched off might either reflect a stable entrainment or the strong urge for a special behaviour that is masked by light, or both. In this context it is worth mentioning that although in DD almost all hamsters exhibited a free-running period ≥ 24 h the previous phase angle of entrainment provided no distinct hint on it, since on average the activity phase began more or less immediately after lights-off. This might, at least partly, be explained by the considerable α decompression at the beginning of DD which is typical for hamsters with access to a RW (personal observation; data not shown), unlike the gradual α decompression under DD in hamsters without wheel. The striking expansion of the activity phase is frequently linked to a massive phase advance of the onset up to several hours, as shown in Fig. 2.1 and Fig. 2.6, and, therefore, supporting the masking hypothesis.

Subsequent to the alternation of the T_a the free-running period (τ) significantly changed in both groups. That is, despite T_a changes in the opposite direction τ consistently tended to become longer. Together with the fact that the number of revolutions per day, but not the period, was significantly different between both groups after the T_a change, the amount of wheel-running activity appeared not to be the decisive parameter for observed τ alterations. Together, several facts are not consistent with the notion of a negative correlation between the activity level and the free-running period (Aschoff 1960, Mrosovsky 1999). Firstly, only few animals (3 out of 17) showed in DD an initial period < 24 h despite pronounced exercise. This distribution of τ values is surely not shifted towards shorter periods compared to hamsters without a wheel. Namely, under comparable conditions in a different study 4 out of 15 animals without RW access exhibited a shorter τ than 24 h (data not shown); and previous studies in which hamsters had only temporarily access to a RW provided evidence for a lengthened τ due to running rather than for the opposite influence (data not shown). In the experiment without RW access mentioned above the exchange of the cages after 20 days in DD was sufficient to alter the period length in some hamsters, pointing to a pacemaker readily adjustable. This would confirm the prevailing notion of a labile pacemaker in Djungarian hamsters (Larkin et al. 2004, Ruby et al. 1996, Steinlechner et al. 2002b).

Secondly, the group that maintained the amount of activity not only changed τ but also significantly increased the free-running period length compared to the second group. Therefore, merely the lengthened period of the latter group in combination with significantly reduced activity follows the prediction. Hence, other factors might be important for the changes of τ. A direct influence of the T_a in fact appears conceivable only in view to the more or less rapid change. Such an abrupt alteration (within a few hours) might serve as an environmental signal strong enough to evoke behavioural responses that in turn may affect the activity rhythm of the animal. Concordantly, visual inspection of the actograms revealed abruptly readjusted τs following the alteration in T_a rather than gradual changes of τ. Due to inconsistency of the results from numerous studies (before), it is perspicuous that the question arose, whether the chosen/common parameter, i.e. total daily activity, is useful for the investigation of the relationship between the ‘level of excitement’ and the pace of the endogenous clock. According to this Koteja et al. (2003) distinguished between total revolutions per day, running speed, and time spent running. The influences of those parameters on τ where compared between a control line of laboratory house mice and a second one that had been selectively bred for high wheel-running activity (Swallow et al. 1998). Mice of the latter line were shown to run approximately twice as many revolutions per day and have a shorter τ compared to controls. Although females ran significantly more than males there was no difference in τ. Within individuals, running speed had generally little effect on τ, and the correlation between time spent on locomotor activity and τ depended on sex. Those and further inconsistent results of the study point to the importance of distinguishing between among- and within-individual effects of locomotor activity on τ, and emphasised the complexity not reducible to a simple causal relationship between the level of activity and the free-running period, which is strongly supported by the present study.

3

The annual activity pattern is stabilised by wheel-running activity

ABSTRACT

Djungarian hamsters (*Phodopus sungorus*) exhibit a pronounced winter acclimatisation with changes in body mass, gonads, fur and thermogenic capacity induced by decreasing day length. To find out to what extent activity rhythms reflect photoperiod-driven seasonality, animals with and without access to a running wheel were exposed to natural lighting conditions (~52° N) and natural ambient temperatures for one year. Registration of locomotion in hamsters with a running wheel revealed a clear activity pattern closely related to dusk and dawn throughout the year. In contrast, animals without running wheel access showed a less stable phase relation between the activity and the day-night cycle in autumn and winter. During this time the activity phase either exceeded the dark phase from dusk until dawn or even became undetectable. This change in the activity pattern correlated with increased locomotion not only during daytime but also per day, especially in autumn. A higher amount of locomotor activity occurred also in hamsters with a wheel, probably reflecting foraging due to increased food hoarding before winter, which is known for several palaearctic hamster species. In a further experiment the phase-reference points lights-off and lights-on within artificial light-dark cycles were compared to sunset and sunrise in an intermediate ratio of light and dark and in long days. With respect to the defined phase-reference points of the zeitgeber the phase relation between activity and the light-dark cycle was similar in natural and corresponding artificial lighting conditions, but dependent on the light-dark ratio, as expected.

INTRODUCTION

The solar day-night cycle provides the most reliable external time cue (zeitgeber) for both daily and seasonal organisation of organisms. Especially animals that have to cope with challenging environmental changes rely on the signal of varying day length. Due to predictability this photoperiodic information allows early enough preparation for seasonal demands, e.g. caused by severe cold and shortage of food in winter. In the strongly seasonal Djungarian hamster (*Phodopus sungorus*) winter acclimatisation already begins in late summer (Steinlechner et al. 1983), whereby low ambient temperatures (T_a) only have a modulating effect on the occurrence of the winter traits (Heldmaier and Steinlechner 1981b, Larkin et al. 2001, Ruf et al. 1993). The diverse physiological and morphological changes such as reduction in body mass, gonadal regression, fur colouration and displaying torpor are driven primarily by decreasing day length (Carter and Goldman 1983, Hoffmann 1973). In European hamsters (*Cricetus cricetus*), in contrast, photoperiodic information is only needed for synchronising their endogenous circannual rhythm (Canguilhem 1989, Masson-Pévet et al. 1994). Thus, decreasing day length is not a requirement for gonadal regression and the change in body weight but for their precise timing. Synchronisation with the rotating earth is controlled by two phases of sensitivity to changes in photoperiod. From mid-November to early April European hamsters respond to long days (Monecke and Wollnik 2004), and around the summer solstice they are sensitive to short days (Saboureau et al. 1999). The latter annual phase has been shown to coincide with a high level of activity and an early activity onset (Monecke and Wollnik 2005). The resulting clear activity rhythm contrasts with the remaining annual activity pattern that is characterised by weak rhythmicity of locomotion in the European hamster (Wollnik et al. 1991). For Djungarian hamsters, lacking an endogenous circannual basis for their seasonality, a daily activity/rest cycle can be expected that is closely related to the essential entraining photoperiod, perhaps only except for the phase of photorefractoriness. During this refractory period in winter the animals 'ignore' the short-day signal and reversion into the summer phenotype is induced. This temporary independence from the photoperiod might be observable in the activity pattern. Besides, it has been shown that the sleep-wake behaviour in Djungarian hamsters is different in short days compared to long days. Light-dark differences are smaller in all vigilance states under short-day conditions at a T_a of

~15°C (Deboer et al. 2000). Therefore, the authors concluded that sleep regulation is uncoupled from the circadian clock in winter-acclimatised hamsters. Thus, in order to see whether on the one hand the activity pattern under short day lengths reflects peculiarities in the behaviour and on the other hand a clear daily rhythm linked to the natural photoperiod during the remaining annual phases is displayed, hamsters with and without access to a running wheel were monitored over one year. In a further experiment we tried to determine whether sunset and sunrise might serve as comparable phase-reference points to lights-off and lights-on in artificial light-dark (LD) cycles for this species.

MATERIALS AND METHODS

Animals and housing

Animal husbandry and all experiments were in accordance with the German Animal Welfare Act. Hamsters were born and raised outdoors under a natural photoperiod (52° 21' N latitude) with natural ambient temperatures. The cages were positioned under a transparent plastic roof but were also exposed to unfiltered daylight. After weaning the animals were housed separately with food (hamster breeding diet, Altromin 7014) and tap water available ad libitum, weekly supplemented by a slice of apple. From the beginning of the experiment hamsters were kept in cages (Makrolon, type III, high) equipped with a wooden nest box and a running wheel (14 cm inner diameter). A magnet attached to the wheels and a reed contact above them enabled registration of revolutions. General activity was monitored with the help of passive infrared (IR) detectors (Conrad Electronic SE, Germany) mounted centrically on the cage lid. Signals were conveyed continuously to a personal computer and stored every 6 minutes. T_a was measured at intervals of one hour with temperature loggers (iButton, Maxim Integrated Products, Inc., Sunnyvale, CA) of different range and resolution for either indoors (DS1921H: 15-46°C, 0.125) or outdoors (DS1921L: −20-85°C, 0.5). Time of day is given as Central European Time (CET).

Data processing and analysis

Consecutive signals of a motion detector were only registered if more than three seconds had elapsed since the preceding signal. This period of refractoriness was applied to compensate for differences in signal output of IR sensors. Due to high sensibility the motion detectors produced a low level of noise which was eliminated for later analyses by raising the base line for general activity from 0 to 10 signals per interval.

Activity onsets and offsets were determined by comparison of raw (running wheels) or slightly processed data (IR detectors; moving average of 1 h) (curve A) and an activity threshold resulting from the same raw data set but processed with a moving average of 24 hours (curve B; lowered by 50% for wheel running). In the time from three hours before sunset until three hours after sunrise the first intersection of both curves followed by $A > B$ was accepted as activity onset, and the last intersection followed by $A < B$ was assessed as offset. Determination was done with self-written software charting actograms with the calculated activity onsets and offsets. Thus, plausibility of the results could be directly checked on inspection. Only few results had to be corrected manually with the help of eye-fitting. Although data resolution corresponded to 6-min intervals mean phase-angle differences (ψ) between activity onsets and lights-off/sunset as well as ψ between offsets and lights-on/sunrise were given in minutes for easier interpretation by the reader.

Activity registration, period analysis (chi-square periodogram; Sokolove and Bushell, 1978) and plotting actograms were based on modified software originally programmed by Thomas Ruf.

Procedures

Experiment I. The experiment comprised of three parts. In the first part (1) 8 Djungarian hamsters (6-7 months old; 4 females, 4 males) were given access to running wheels near the winter solstice (2003). One female hamster avoided the wheel and was replaced after nine days. Another female animal had to be removed from the experiment after 167 days, and one male hamster died after 284 days. In the second part (2) young male hamsters (5-8 weeks old; $n = 4$) gained access to a running wheel before the summer solstice (2004) for comparison of age-effects. For the third part (3) young male hamsters (7 weeks old; $n = 4$) obtained access to a running wheel again at the summer solstice (2005), and 4 male siblings were kept under the same conditions except for the running wheel access.

Experiment II. Two groups of male hamsters were either kept under LD 16:8 (L: ~300 lx, D: < 2 lx; $n = 4$, 8.5-11 months old) or LD 12:12 (L: ~300 lx, D: < 0.1 lx; $n = 8$, 11-16 months old). After registration of wheel running under the artificial light-dark cycle (LD_{art}) with a rectangular light signal the animals were exposed to natural lighting conditions (LD_{nat}) of similar photoperiods (from sunrise until sunset) compared to LD_{art}, respectively; i.e. hamsters were either transferred from LD_{art} 16:8 (lights on 0500-2100 h; T_a 25 ± 1°C) to LD_{nat} 16.8:7.2 (daytime 0400-2047 h; T_a range 15.5-28°C) on 19 June or from LD_{art} 12:12 (lights on 0600-1800 h; T_a 21 ± 2°C) to LD_{nat} 11.9:12.1 (daytime 0615-1809 h; T_a

range 2.5-17.5°C) on 27 September. T_a ranges given for natural conditions (see above) are related to analysed days. For determining activity onsets and offsets ten consecutive days from LD_{art} and LD_{nat} (15 days for LD_{nat} 16.8:7.2) were used, respectively. The first week after transfer to natural conditions was excluded from the analysis.

RESULTS

Experiment I. All animals with access to a running wheel exhibited a clear annual activity pattern with wheel running almost completely restricted to the night (Figs. 2.1A; 2.2A; 2.3A). Under short days the activity phase (α) was more fragmented and small amounts of wheel running occurred during the daytime. In two female hamsters the distribution of nocturnal wheel-running activity was slightly different compared to the remaining individuals. Under short-day conditions both animals showed an unsteady pattern (Fig. 3.1A) instead of a bimodal pattern with the main activity proportion in the first half of the night and a second peak at the end. This is clearly observable in Fig. 3.7B. Analysis of the phase relation between the activity onset and sunset revealed that wheel running in male hamsters began earlier throughout the year compared to females (33 ± 2 min; Fig. 3.1B, C). However, the seasonal change of ψ_{on} was similar for both groups exhibiting the earliest onsets during summer and the latest onsets in winter with respect to sunset, and a gradual transition in-between (Fig. 3.1B, C). This gradual change of ψ_{on} in the course of the year is also evident in the results in the other two parts (Figs. 3.2B; 3.4), even though less pronounced in those of part 2 (Fig. 3.2B). The latter is attributable to one activity record (Fig. 3.2A, right) as well as the distinctly delayed mean offsets during autumn and winter (Fig. 3.2B) compared to parts 1 and 2 (Figs. 3.1B, C; 3.4, upper graph). In general, variability in mean onsets is considerably lower compared to mean offsets. In this regard comparison of actograms and mean offsets suggests that in some cases the upper (later) limit of the range might be more meaningful than the mean value. In fact late wheel-running activity bouts at the expected end of α seem to be representative although they were often lacking. If this is taken into account results of part 1 indicate that the end of nocturnal wheel running tracked the beginning of civil twilight (Fig. 3.1B, C), which is confirmed by the findings of part 2 and 3 at least for the summer (Figs. 3.2B; 3.4A).

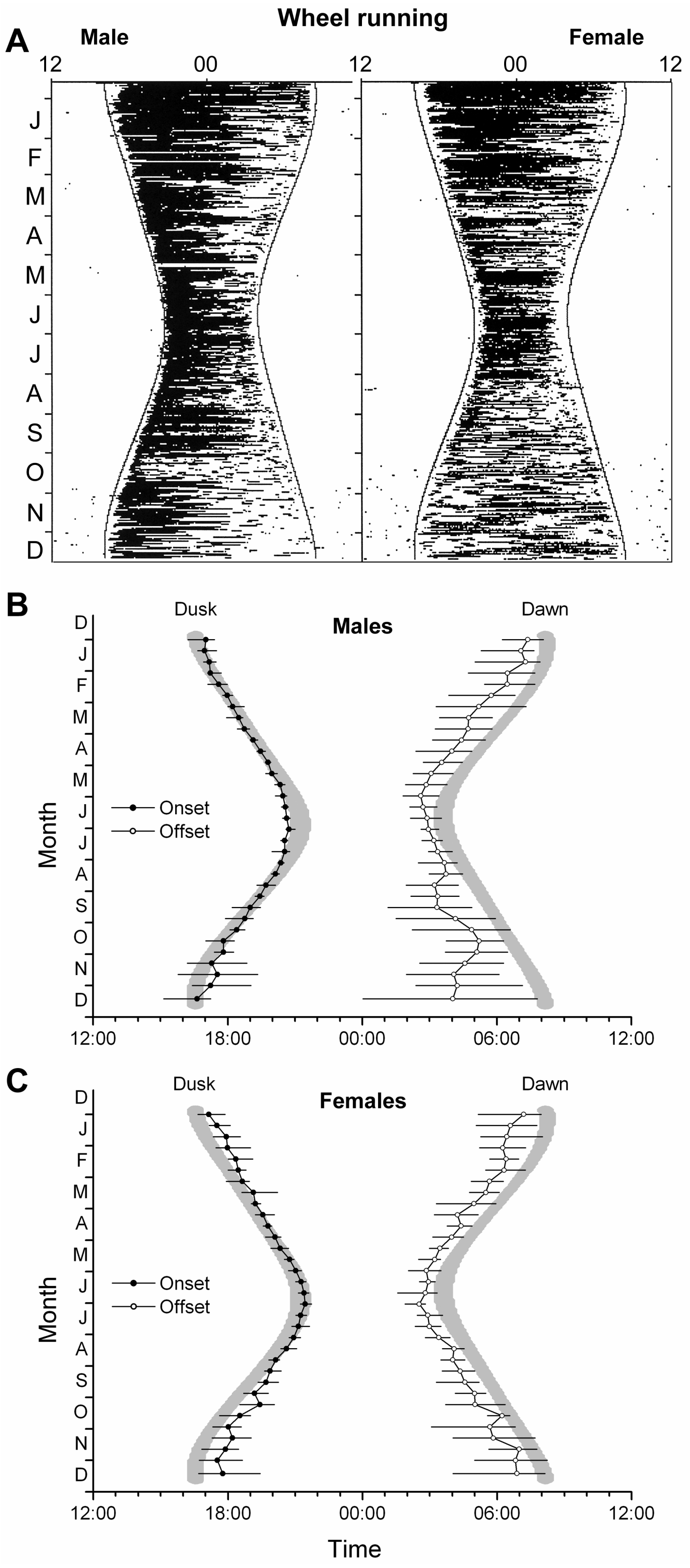

Figure 3.1. Actograms (A) of two individuals over one year beginning at the winter solstice. Sunrise and sunset are indicated by lines. Wheel running onsets and offsets (mean and range of 11 days) from male (B) and female (C) animals (*n* = 3-4, each).

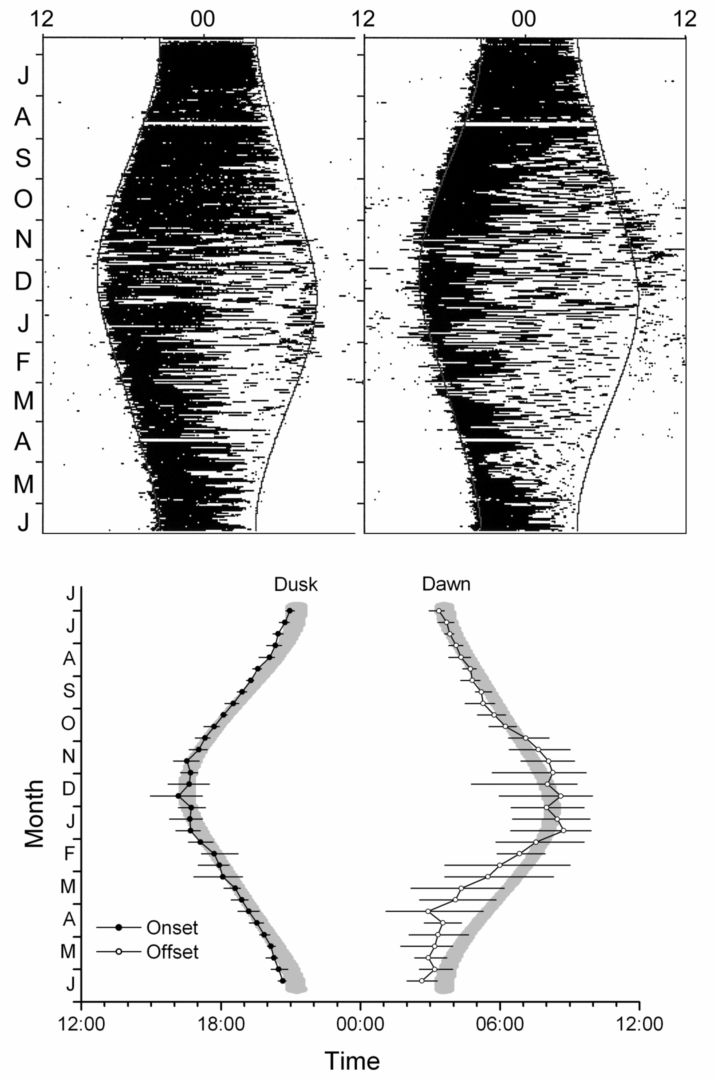

Figure 3.2. Actograms (A) of two individuals over one year beginning at the summer solstice. Sunrise and sunset are indicated by lines. Wheel running onsets and offsets (mean and range of 11 days) of 4 hamsters (B).

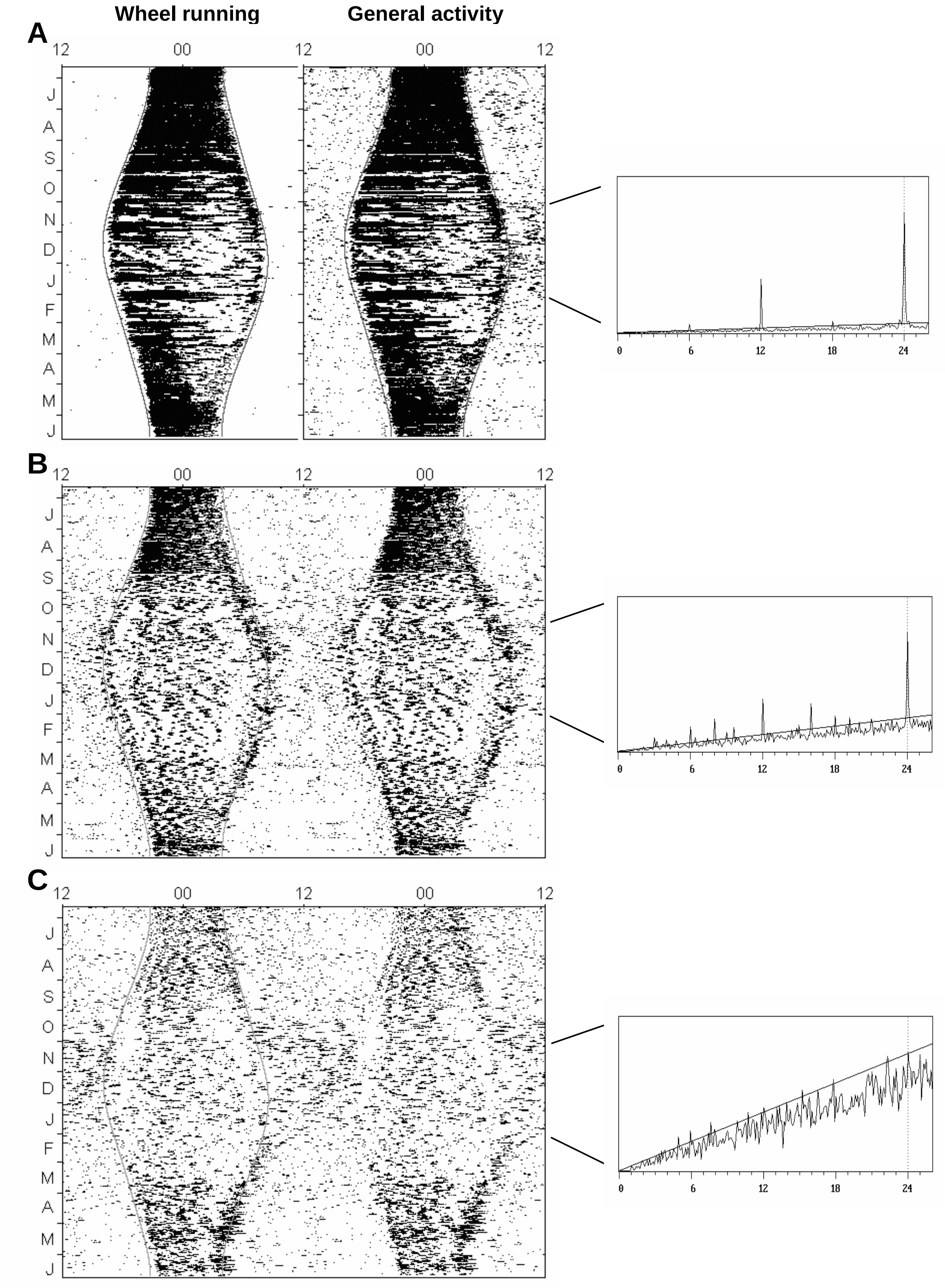

Figure 3.3. Actograms of one individual with access to a running wheel (A) and two hamsters without a wheel (B, C; double plots). Sunrise and sunset are indicated by lines. Rhythmicity of activity from November until January is shown in periodograms (right).

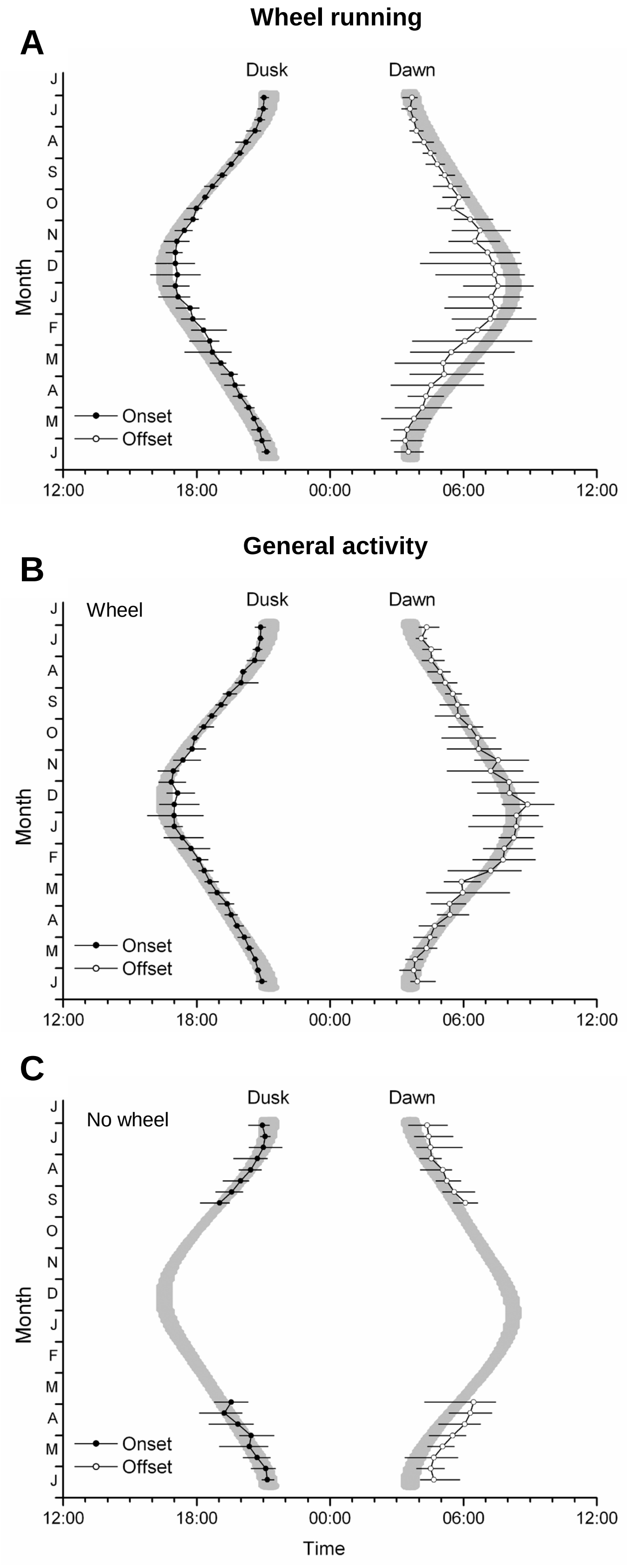

Figure 3.4. Activity onsets and offsets (mean and range of 11 days) of hamsters with access to a running wheel (*n* = 4) for both wheel running (A) and general activity (B). (C) Onsets and offsets of hamsters without running wheel access (*n* = 4).

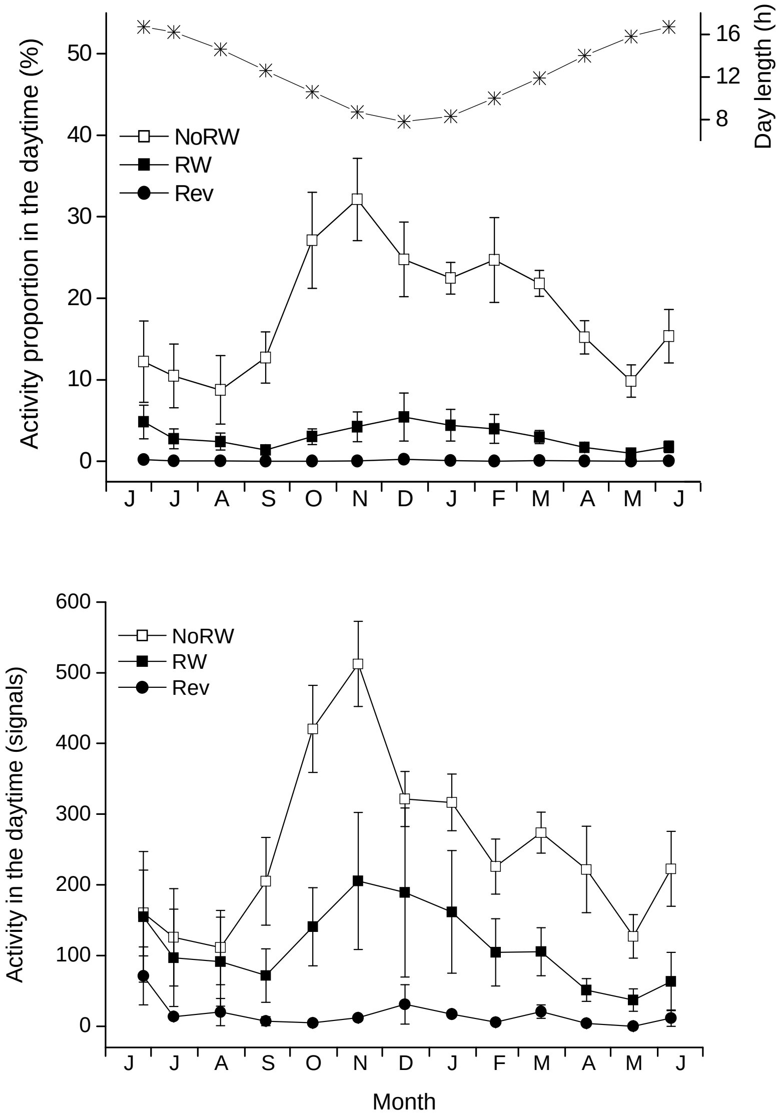

Figure 3.5. Activity during the photophase (monthly mean ± SEM) of hamsters with and without access to a running wheel (*n* = 4, each group). General activity is represented by IR signals (squares) and the amount of wheel running is given as wheel revolutions (circles).

In the third part of experiment I, mean activity onsets in summer were more related to sunset compared to the situation in winter when onsets were locked on the end of civil twilight (dusk). This seasonal change of ψ_{on} in wheel-running activity (mentioned above) is almost congruent with the findings for general activity (IR signals) of the same individuals. However, the mean onsets were partly very slightly advanced (8 ± 1 min) compared to the onsets of wheel running (Fig. 3.4, upper graphs). Until autumn and during spring the mean offsets of wheel-running activity tracked the beginning of civil twilight (dawn), whereas offsets of general activity, in comparison, were delayed throughout the year (42 ± 3 min) and related to sunrise until late winter followed by a slight phase advance. Concerning wheel running the offsets were slightly advanced under short photoperiods until spring, which, in connection with delayed onsets, led to shortened activity phases relative to sunset and sunrise. Comparison between the annual pattern of general activity and wheel running, which was part of general activity, showed that running-independent activity was mainly displayed at the end of α (Figs. 3.4A, B; 3.7, see arrows).

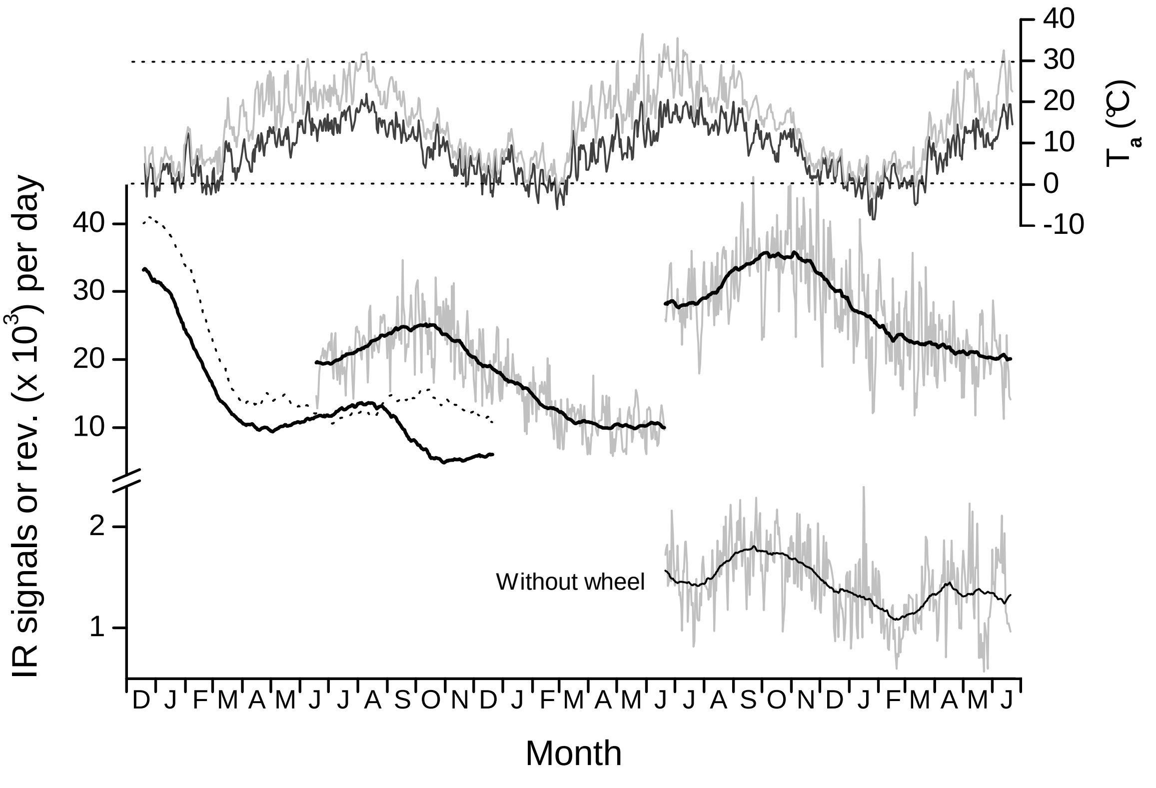

Figure 3.6. Mean daily wheel-running activity as raw data (grey line) or as moving average (50 values) of male (bold black line) and female (dotted line) hamsters. General activity (IR signals) of hamsters without a wheel is also shown as raw data (grey line) and as moving average (thin black line). The ambient temperature is given as daily maximum (grey) and minimum (black) value.

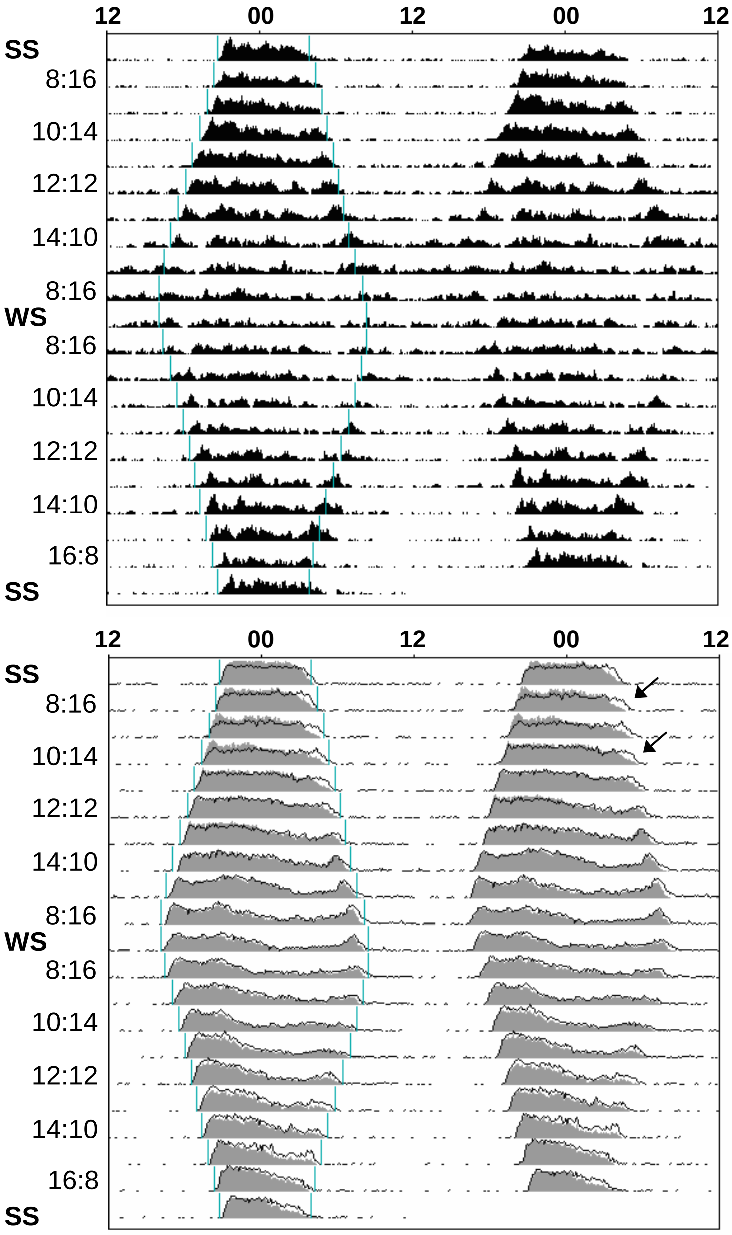

Figure 3.7. Activity pattern (mean of 7 days around the given photoperiod) of hamsters without a wheel (upper graph) and with running wheel access (lower graph). For the latter group both general activity (black line) and wheel running is illustrated. Sunrise and sunset are shown with vertical lines. General activity independent of wheel running is indicated by a considerable difference between both activity levels (arrows). SS = summer solstice, WS = winter solstice.

Activity data of hamsters without access to a running wheel provided reliable onsets and offsets only under long days due to two individuals that showed very weak rhythmicity during autumn and winter (Fig. 3.3C). In summer and spring of the following year the animals began their nocturnal α (mean onset) between sunset and the end of civil twilight, i.e. during dusk. The mean offset locked on sunrise in summer and was markedly phase-delayed during the following spring. Analysis of activity during daytime revealed marked differences between hamsters with and without access to a running wheel (Fig. 3.5). The proportion of activity in the daytime was considerably increased throughout the year in animals without a wheel with the highest values in autumn and winter. For absolute amounts of activity a similar annual course was found. Both groups exhibited most daytime activity in autumn and winter, but from September until the end of the experiment hamsters without a wheel were more active during the photophase than animals with access to a wheel. The daily amount of wheel revolutions (rev./d) continuously decreased from December until April in both males and females of part 1 (Fig. 3.6). This course applies to the findings of both other parts (2 and 3) as well, albeit with a lower amplitude. In the latter parts the highest numbers of rev./d were found in September and October. The annual course of the amount of activity in hamsters without wheel was similar compared to exercising animals except for the slight second increase beginning in February.

Experiment II. After transfer from LD_{art} to LD_{nat} the number of wheel turns under long-day conditions (13,792 ± 1,478 vs. 20,343 ± 1,173 rev./d; $n = 4$) was not significantly increased. Under short-day conditions locomotion also remained unchanged after the transfer (18,681 ± 3,104 vs. 16,863 ± 2,450 rev./d; $n = 8$).

Phase-angle differences of activity onsets (ψ_{on}) and offsets (ψ_{off}), respectively, were similar when comparing LD_{art} and LD_{nat} under long-day conditions (ψ_{on} -10.8 ± 6.3 vs. -8.5 ± 8.3 min; ψ_{off} 36.3 ± 7.3 vs. 39.9 ± 4.3 min) as well as under short-day conditions (ψ_{on} -37.7 ± 3.9 vs. -38.6 ± 4.3 min; ψ_{off} 99.2 ± 11.7 vs. 86.9 ± 10.4 min; Fig. 3.8). Comparison of long-day and short-day conditions revealed that in the latter case activity onsets were delayed and offsets were advanced ($P < 0.01$; Mann-Whitney rank sum test).

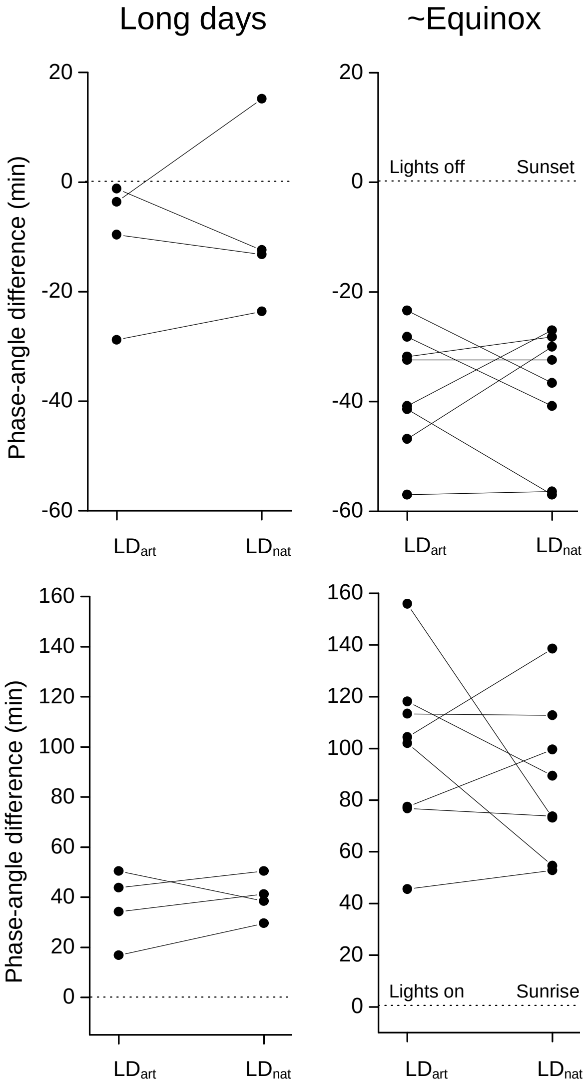

Figure 3.8. Mean activity onsets (upper graphs) and offsets before and after a transfer from an artificial LD cycle (LD_{art}) with abrupt transitions to natural lighting conditions (LD_{nat}) with a similar LD ratio with respect to sunset and sunrise.

DISCUSSION

Seasonal changes in phase and duration of daily activity have been shown for various species (Aschoff 1969, Daan and Aschoff 1975, Hut et al. 1999, Saboureau et al. 1979). In their extensive work Daan and Aschoff (1975) pointed out general characteristics of the annual activity pattern consistent for birds and both nocturnal as well as diurnal mammals. As expected, there is a strong correlation between activity time (α) and day length, either positive in day-active animals or negative in night-active species (see also Fig. 3.9). However, animals follow changes of the photoperiod only to a certain extent. Thus, compression and extension of α are limited, which was impressingly demonstrated under extreme photoperiodic conditions at the Arctic Circle. Seasonal changes of ψ_{on} and ψ_{off} displayed a converse pattern which provided a further argument for the postulated two-oscillator model (Daan and Aschoff 1975, Pittendrigh and Daan 1976) that is still under discussion (Daan et al. 2001, 2005).

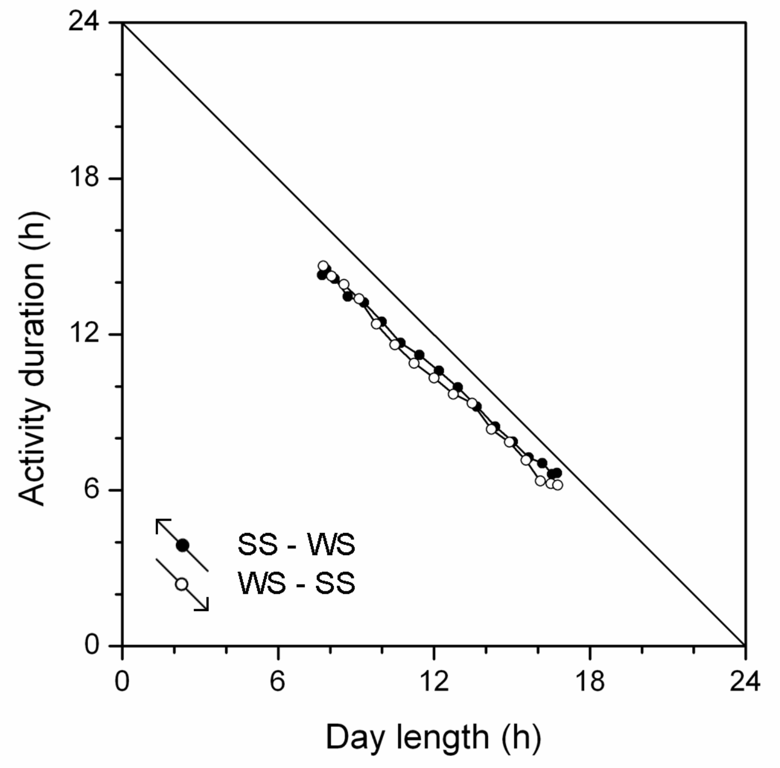

Figure 3.9. Activity time (α) as a function of day length for hamsters with access to a running wheel (*n* = 4). Mean values of 11 days, respectively, are shown for one year, i.e. from the summer solstice (SS) until the winter solstice (WS) with decreasing photoperiod and vice versa.

The present findings in Djungarian hamsters (*Phodopus sungorus*) with access to a running wheel are well in accordance with the previous results of wheel-running activity in night-active rodents (*Mesocricetus auratus*, *Glaucomys volans*; Daan and Aschoff 1975) monitored at a temperate latitude (~48° N). Relative to sunset the locomotor activity began earlier during summer compared to winter, whereas comparable seasonal changes in the phase relationship between the end of activity and sunrise were not found. As a consequence the negative correlation between the duration of daily activity and the corresponding day lengths revealed a slope slightly different from –1 (Fig. 3.9), confirming the results in Syrian hamsters and flying squirrels. Both species were also monitored at the Arctic Circle (~66° N) with extreme seasonal changes in photoperiod, revealing the typical S-curve of activity time as a function of day length, also shown for day-active tree shrews (*Tupaia belangeri*) and various birds (Aschoff 1969, Daan and Aschoff 1975). The flattened slope at the ends of this S-curve reflects the decreased entrainment capacity of extremely long and short photoperiods. Unlike the results from the temperate latitude not only ψ_{on} but also ψ_{off} in Syrian hamsters and flying squirrels shows a pronounced seasonal change at the higher latitude with a mirrored course compared to the onset, i.e. similarly contributing to the S-curve. Similar findings would be likely for Djungarian hamsters but have to be proven at extreme latitudes.

Generally, the onset of activity was timed to dusk when the rate of the change in light intensity is highest, thus providing a reliable signal whose strength is only marginally influenced by cloudiness. Due to the latter, together with the fact that no other properties of the entraining oscillator are involved such as the ratio of day and night or the amplitude of light intensity, Daan and Aschoff (1975) supposed a direct effect by light on the activity rhythm (masking; for review, see Mrosovsky 1999) rather than resetting of the endogenous clock. This effect, for instance, is missing in burrowing European ground squirrels (*Spermophilus citellus*) which 'renounce' civil twilight information. Instead, they generate their own pattern of exposure to light (Hut et al. 1999). However, ground squirrels make use of a circannual 'programme' unlike the Djungarian hamster which is known for its photoperiodism. Therefore, it is likely in the case of burrow-dwelling Djungarian hamsters that this major light signal plays a crucial role. An activity-suppressing influence (negative masking) that is more effective for wheel running than for general activity would explain the more stable annual activity pattern in hamsters with access to a running wheel. The almost complete lack of wheel-running activity from sunrise until sunset in this study strongly suggests a light-induced masking effect. However, comparison of the wheel running onset with the corresponding onset of general activity, shown in Fig. 3.4, revealed that the latter was only marginally advanced. This approximate temporal congruency probably reflects a dominant contribution of the entrained clock to the temporal occurrence of the activity onset (Daan and Aschoff 1975), confirmed by mean onsets with a similar phase relation in hamsters without a wheel. The situation is different for the end of activity which is also shown in Fig. 3.4. In running wheel hamsters the mean offset of wheel running was always advanced compared to the end of their general activity. Thus, wheel running might have been suppressed by light before the clock-influenced end of α, which is supported by a similar ψ_{off} during summer of hamsters without running wheel access.

The general tendency of increased activity during daytime in autumn and winter was especially prominent in animals without a wheel combined with an activity rhythm less clear than during summer and spring. On the basis of the two-oscillator model for the endogenous clock, comprising a morning (M) and evening (E) oscillator (Pittendrigh and Daan 1976, Daan et al. 2001, Steinlechner et al. 2002a), a decreased coupling due to a reduced zeitgeber strength in short days is imaginable, especially in this species (Steinlechner et al. 2002b). For instance, reduced capability to reentrain was shown with a phase delay of the LD cycle causing free-running or even arrythmicity (Ruby et al. 1996). Likewise, short light pulses induced arrhythmicity of activity, body temperature and pineal melatonin secretion despite the enduring LD cycle, which argues for a labile pacemaker in Djungarian hamsters (Steinlechner et al. 2002b). In this regard, it should be mentioned that in the two-oscillator system light might interfere with both the mutual interaction of the M/E oscillators and their coupling with dawn/dusk, respectively.

In general, there is no doubt about the potential of wheel-running activity to influence the circadian system (reviewed in Mrosovsky 1996). Photoresponsiveness, however, obviously was not perturbed in the present study since the winter traits occurred also in hamsters with weak (or even absent) behavioural rhythmicity (part 3), indicating an entrained clock still driving the essential pineal melatonin secretion (Bartness and Goldman 1988, Carter and Goldman 1983, Darrow and Goldman 1985). This kind of incongruity of the overt rhythm and the underlying oscillation relates to findings that indicate a sleep-wake cycle uncoupled from the clock under short-day conditions (Deboer and Tobler 1996, Deboer et al. 2000, Palchykova et al. 2003). Hence, together with the fact that the tendency of activity shifted into daytime in short days was also apparent in hamsters with a wheel, albeit much less pronounced compared to animals without a wheel, in the current study a masking effect is suggested. Even so, whether wheel running caused a more stable activity pattern, due to negative masking by light or a feedback to the pacemaker or a combination of the two, remains unclear.

The activity maximum in September and October might reflect foraging due to increased food hoarding which is described for several palaearctic hamster species for the time of August until November, correlated with increased activity during daytime (Flint 1966). A general tendency of considerably decreased wheel running after one year argues for a reduced attractiveness of the wheel and/or an age effect on extensive exercise. Consistently, hamsters without running wheel access again increased their activity during spring shown in Fig. 3.6. Translating these results into natural incidents, however, has to occur carefully as little is known about the natural behaviour and light perception of this burrow-dwelling species.

Due to simplicity and thus reproducibility most experiments in rhythm research are still carried out under artificial LD cycles with rectangular transitions. The appropriate capacity of dawn and dusk to entrain daily rhythms has been intensively discussed (Aschoff 1960, Daan and Aschoff 1975), and increased entrainment properties of twilight compared to abrupt light transitions have been shown (Boulos et al. 2002, Boulos and Macchi 2005), supporting its crucial role in environmental synchronisation. One problem still remains in comparing natural LD cycles (or their simulation) and artificial rectangular LD cycles, namely, to ensure equivalent durations of the light and dark phase, respectively (Boulos et al. 1996). Therefore, in a further experiment we tested whether the arbitrarily selected phase-reference points, sunset and sunrise, coincide with lights-off and lights-on in a comparable LD cycle. For the selected conditions we found no differences between ψ_{on} and ψ_{off}, respectively, when compared for LD_{art} and LD_{nat}, neither under long-day conditions nor under the intermediate ratio of light and darkness, supporting the use of sunset and sunrise as phase markers. However, significance of the present results is limited to the given light intensity since this factor was shown to influence the phase relation between a zeitgeber and the entrained rhythm. A more extensive approach with several different LD ratios in combination with varying light intensities and a constant T_a would be helpful in answering this question.

4

Wheel running affects seasonal acclimatization of physiological and morphological traits

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ABSTRACT

Wheel running was previously shown to influence body mass and torpor in short day-acclimatized Djungarian hamsters (*Phodopus sungorus*). To determine whether the exercise-induced effect on body mass depends on the annual phase, hamsters were exposed to the natural change in photoperiod and given access to a running wheel (RW) either before, in the middle of, or at the end of the descending body mass trajectory during seasonal acclimatization. Due to wheel running the seasonal weight cycle was prevented or aborted by abruptly rising body mass, resulting in a weight appropriate for summer despite exposure to short days. Torpor was inhibited and testicular recrudescence was advanced compared to controls. In contrast, the change into winter fur remained unaltered. Analysis of body composition and plasma leptin revealed a low body fat mass in RW hamsters not only in winter but also in summer, suggesting a lack of seasonal adiposity. Chronic leptin infusion in winter only decreased body mass in RW individuals although their relative body fat mass probably was even lower than in sedentary hamsters. A constantly low body fat mass is conceivably reflecting an exercise-dependent change in metabolism, consistent with increased bone mineral content and density in RW hamsters. Additionally, bone area was increased, again supported by elongated vertebral columns. Together, the results show a striking effect of wheel running on body composition and the seasonal pattern of body mass and they suggest that the photoperiodic regulation of body mass is regulated differently than the reproductive and pelage responses.

INTRODUCTION

Voluntary wheel-running activity still baffles scientists although the first revolving drum for rodents was applied more than one century ago (Stewart 1898). For a long time this monotonous locomotion was related to natural behaviors like exploratory migration (for review, see Mather 1981) and, indeed, at first view wheel running can be imagined to substitute covering distances in search of food and mate. By others, in contrast, this activity is considered self-reinforcing and an artifact of captive environments or of the running wheel itself without any equivalent in the natural behavior pattern (for review, see Sherwin 1998b). Nevertheless, registration of wheel running is still a standard tool for determination of activity rhythms in chronobiology. In this regard it seems to be necessary to know potential side-effects of this special kind of running behavior. In Syrian hamsters (*Mesocricetus auratus*), contrary to intuitive expectation, extensive locomotion leads to a massive increase in body weight after several weeks with access to an activity disc or a running wheel (Borer 1974, Gattermann et al. 2004). Weight gain, however, is not a result of increased fat storage (Borer and Kooi 1975) due to excessively heightened appetite because body composition remains unaltered (Gattermann et al. 2004). Instead, running stimulates growth which was shown first by increased body lengths (Borer 1974) and later strongly supported by bone measurements on radiographs (Borer and Kuhns 1977) as well as elevated growth hormone levels in exercising individuals (Borer and Kelch 1978). According to this running-induced impact the higher body weight was shown to be defended after a temporary food restriction (Borer and Kooi 1975). Also in Syrian hamsters photoperiod-dependent gonadal atrophy is reduced (Gibbs and Petterborg 1986) and hibernation is inhibited (Menet et al. 2003) due to running. However, neural integration of photoperiodic time measurement is not prevented (Menet et al. 2005). That is, although information about changing day lengths is conveyed to the responsible neuronal structures the development of the corresponding phenotype is modulated. In this respect seasonal acclimatization in the Djungarian hamster (*Phodopus sungorus*, also known as Siberian hamster) provides an appropriate animal model to study the physiological and morphological consequences of running exercise. The adaptation to the strongly seasonal habitat impressively appears when the animals turn into their winter state. They change the fur color, reduce their body mass, exhibit gonadal regression leading to reproductive quiescence, and finally display torpor (Figala et al. 1973, Hoffmann 1973). Due to refractoriness to the elongated nocturnal release of melatonin by the pineal gland short-day acclimatization reverses in late winter (Hoffmann 1973, Steinlechner et al. 1983) or after at least 20 weeks in artificial short days (SD) (Hoffmann 1978, Tups et al. 2006).

An early study about influences of exercise on body weight in *Phodopus sungorus*, where SD was mimicked by melatonin injections, did not reveal effects comparable to those shown previously in Syrian hamsters (Bartness and Wade 1985). In contrast, Freeman and Goldman (Freeman and Goldman 1997) demonstrated that a laboratory-selected line of photoperiod nonresponsive hamsters (*P. sungorus*) will exhibit winter-type responses following SD exposure only when they are given free access to a running wheel. The first evidence of exercise-induced perturbations of seasonal acclimatization in the Djungarian hamster was provided by Thomas et al. (1993) showing that castrated male individuals increase their body weight under SD conditions when given access to a running wheel. Furthermore, the animals rarely displayed torpor whereas the change of the pelage color was not altered. These striking findings, however, apparently went unnoticed so that despite the remarkable potential of wheel running to perturb a program evolved for survival in a harsh climate only little is known about the effects and even less about the underlying mechanisms. More recent studies on the same species suggested a role for the intergeniculate leaflet of the thalamus (IGL) in the mechanism by which wheel-running activity can modulate photoperiodic responsiveness (Freeman et al. 2004a, 2006). The aim of the present study was to characterize how running exercise alters the seasonal acclimatization of physiological and morphological traits triggered by natural changes of photoperiod and ambient temperature. Therefore, hamsters were given access to running wheels at different stages of the seasonal cycle to see whether the effect of exercise on body weight and other seasonal traits depends on the phase of the annual rhythm. Physiological and morphological changes were studied by analyzing gonadal development, responses to chronic leptin infusion, and body composition in view of the seasonal change in adiposity associated with the body weight cycle in this species.

MATERIALS AND METHODS

Animals and housing

Animal husbandry and all experiments were in accordance with the German Animal Welfare Act (ref.: 05/1061). Hamsters were born and raised outdoors, thus exposed to the natural changes in photoperiod (52° N latitude) and ambient temperature. After weaning they were housed singly with food (hamster breeding diet, Altromin 7014) and tap water available ad libitum, supplemented by a slice of apple once a week. Hamsters with access to a running wheel (14 cm inner diameter) (RW) were transferred into larger cages (Makrolon, type III, high), whereas control animals (sedentary) remained in standard cages (Makrolon, type II). In the running wheel cages a wooden nest box was available throughout the experiment. Sedentary hamsters kept in standard cages were also provided with a wooden nest box (experiment 1) or soft paper tissues for nest-building (experiment 2 and 3). Body mass data were used for calculation of group means only if all measurements were taken within two consecutive days.

Procedures

Experiment 1. Near the winter solstice 4 female and 4 male Djungarian hamsters (6-7 months old) were moved to RW cages. One female hamster avoided the wheel and was replaced after 9 days. Another female animal had to be removed from the experiment after 167 days. Other 4 female and 2 male hamsters (6-7 months old) remained in type II cages without a running wheel.

Experiment 2. Before the summer solstice 4 male hamsters (5-8 weeks old) were transferred to RW cages, whereas 8 male individuals (6-8 weeks old) were kept in standard cages without a wheel. The fur color index (for definition see below) was determined once at the end of December and at the beginning of January.

Experiment 3. About 3 weeks after fall equinox, during transition into the winter phenotype, 8 male hamsters (5-7 months old) were transferred into cages equipped with a running wheel. The control group also consisted of 8 male hamsters (5-7 months old) kept in small cages without a wheel. One RW individual died several weeks before the end of the experiment reducing sample size for fat extraction to $n = 7$.

Fur color index

Molting from a grayish brown fur in summer to a white winter fur was determined according to the six stages defined by Figala et al. (1973). The final stage was established in December.

Torpor

Events of daily torpor were determined by visual inspection and handling when animals were weighed. Since torpor bouts usually begin in the morning and may last for about 8 hours, weighing was timed (usually 1:00 pm; range 12:00-3:00 pm CET) to minimize the possibility of overlooked events. Determination is relatively easy due to lowered body temperature and breathing rate, typical posture, closed eyes despite disturbance, and sluggishness of torpid hamsters.

Implantation and leptin treatment

Recombinant mouse leptin (R&D Systems Ltd., Germany) was dissolved in 15 mM HCl and adjusted to pH ~5.2 with 7.5 mM NaOH for administering 15 µg per day per animal via osmotic mini-pumps (Alzet, model 1007D). Gelatine capsules of a similar size filled and coated with silicone served as dummies for sham treatment. In mid-December mini-pumps and capsules were implanted subcutaneously in the scapular region under isoflurane anesthesia. They were replaced after 7 days. Thus, the animals implanted with mini-pumps received leptin for about 14 days. During this period all individuals were weighed every day. Mini-pumps and dummies were removed after about 6 weeks.

Blood samples

Blood samples were obtained under isoflurane anesthesia by retroorbital sinus puncture with heparinized micro-hematocrit tubes just before implantation of the osmotic mini-pumps and dummies. After centrifugation plasma was stored at –20°C for later leptin analysis.

Leptin assay

Bound leptin in plasma was measured by a specific radioimmunoassay which was directed to human leptin. For details see Lewandowski et al. (1999) and Horn et al. (1996). The antibody is directed towards a region of leptin which is highly conserved between species (Brabant et al. 2004, Nave et al. 2003). Previous studies had confirmed that the antibody provides serum dilution curves with parallelism to the standard curve suggesting the equivalence of leptin determinations in the hamster and thus confirming the suitability of the approach.

Testis palpation

According to Hoffmann (1973) four different testis sizes were distinguished: Stage 0 (not palpable), stage 1 (small), stage 2 (medium size), stage 3 (large). The initial palpation was carried out in the middle of December (14th/15th) when the first mini-pumps and dummies were implanted. From the beginning of January (6th) onwards the testes were palpated weekly under slight isoflurane anesthesia.

Dual-energy X-ray absorptiometry

In February body composition was analyzed under isoflurane anesthesia with DEXA (PIXImus2 scanner, software version 1.46.007, GE Medical Systems, Madison, Wisconsin, USA). The head of the animals was excluded from the measurement. Bone mass is not included in DEXA-provided values for total body mass (head excluded) and lean mass. Food was removed from the cages about 4-5 hours before the procedure. Lengths of the femora and the vertebral columns between head and pelvis were measured on radiographs of the animals' dorsal side. The bodies were sprawled and the limbs were abducted. To measure the femora a box was drawn and adjusted to the bones with the software CorelDRAW 12. The length of the box corresponded to the straight line between both ends of the femur, thus comparable to measurements with calipers. Values were averaged for each animal. For measurements of the vertebral columns a plug-in (plotcalc) for CorelDRAW was used providing the length of drawn curves.

Fat extraction

In June body composition was determined by fat extraction. About 3-6 hours prior to analysis food was removed from the cages. Under deep isoflurane anesthesia the heart of the animals was excised. Subsequently, heart and testes were weighed and the bladder was emptied. Body weight of the almost exsanguinous carcasses was determined again (basic value) before storing at –20°C for later extraction. For this the carcasses were minced and freeze-dried to a constant weight. After fat extraction with petroleum ether for 6 hours in a Soxhlet apparatus the solvent was removed and the fat was weighed. In a second step the dried material was heated together with hydrochloric acid. Remaining fat in the filtrate was analyzed as described above.

Statistical analysis

The effect of wheel-running activity on body mass as well as the effect of leptin treatment on body weight were evaluated using repeated measures analysis of variance (ANOVA), with group (RW vs. control or leptin vs. no leptin) and time elapsed (e.g. day of treatment) as factors. When interaction terms (group x day) were significant the Tukey test for multiple comparison was applied. For comparison of two unpaired samples the Mann-Whitney rank sum test was used. Paired samples were compared with the Wilcoxon signed rank test. Analysis of Covariance (ANCOVA) was used to compare linear regressions. The quality of relation between data is shown by correlation coefficients (Pearson). Differences were considered significant when $P < 0.05$. Data are given as mean ± SEM. Statistical procedures were made using Statistica 6 (StatSoft Inc., Tulsa, Oklahoma).

RESULTS

Experiment 1. In late summer all hamsters started to respond to the decreasing day length by reducing their body mass. Around the winter solstice when body weight reached the nadir one group (RW) gained access to running wheels. Thereupon the body mass abruptly increased and reached a high summer level within about 7 weeks, i.e. at the beginning of February. Conversely, the control animals still retained their low short-day body weight for a further 6-7 weeks before exhibiting the spontaneous gradual rise which is generally completed in summer (Fig. 4.1A).

Experiment 2. Running wheels were available for one group of young hamsters from June onwards. In the course of the following year the sedentary group, i.e. without wheel, showed the normal annual cycle in body mass (Fig. 4.1B). The group with access to a running wheel failed to reduce the body mass. Instead, the individuals generally gained weight continuously until the following summer. There was no significant difference in the final stage of changed fur color between exercising (median 4; range 3-4) and sedentary hamsters (4; 3-5).

Experiment 3. Access to a running wheel during the transition into the winter state inhibited a further decline in body mass (Fig. 4.1C). Instead of a continued reduction of body mass expected for the normal photoperiodic response of Djungarian hamsters, the individuals with wheels rapidly regained body mass, reaching a significant mass elevation within 12 days (RM ANOVA; $F = 28.1$; $P < 0.001$; Tukey). The sedentary hamsters, until May, had completed their normal seasonal rise in body mass, but did not attain the mean mass of the RW hamsters. Body mass data from the leptin experiment as well as the last seven measurements with reduced sample size in the RW group were excluded from the statistical analysis above.

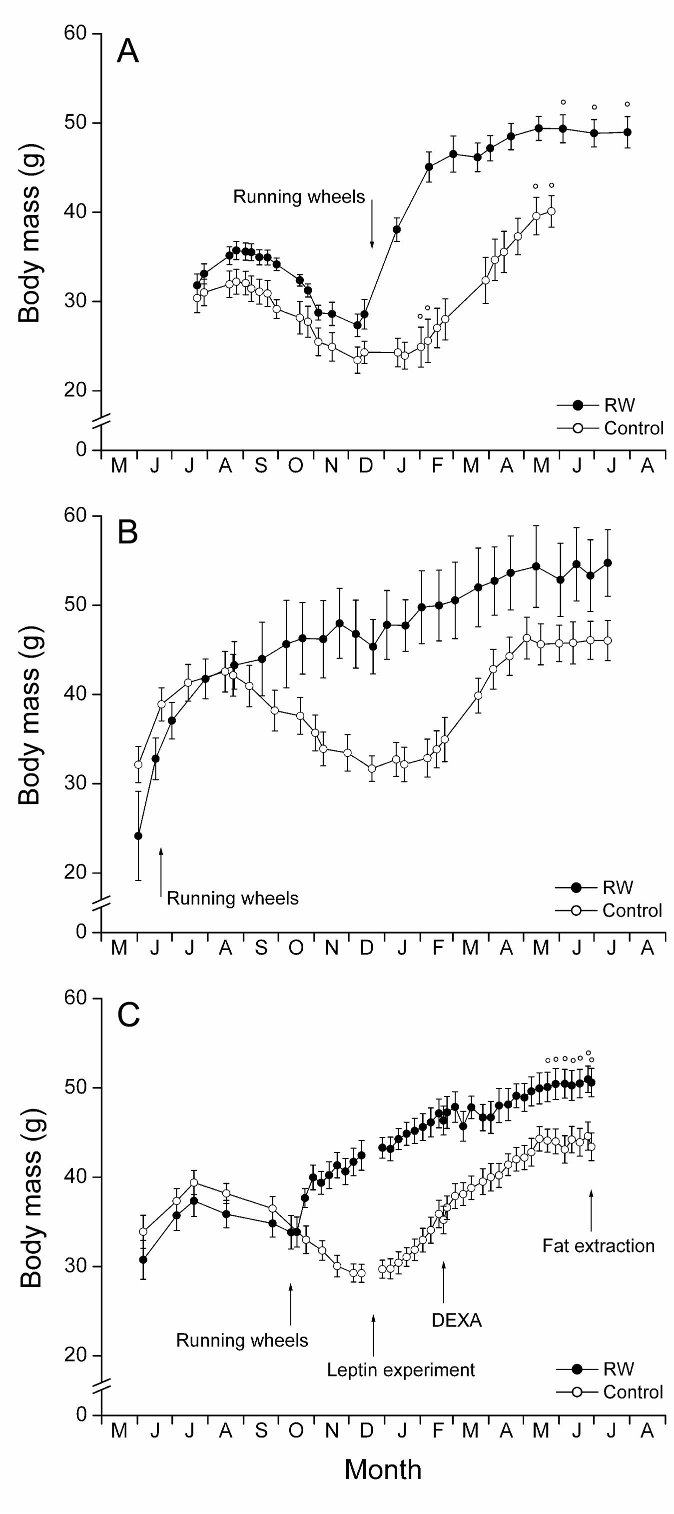

Figure 4.1. Effect of wheel running on the seasonal body weight cycle. *A*: One group (*n* = 8/°7) had free access to running wheels from December 19th. The control group (*n* = 6/°5) was kept without wheels. *B*: Running wheels were available for one group (*n* = 4) from June 18th whereas the second group (*n* = 8) had no access to wheels. *C*: From October 12th one group (*n* = 8/°7) had access to running wheels. Control animals (*n* = 8) were housed permanently without a wheel. Data are means ± SEM.

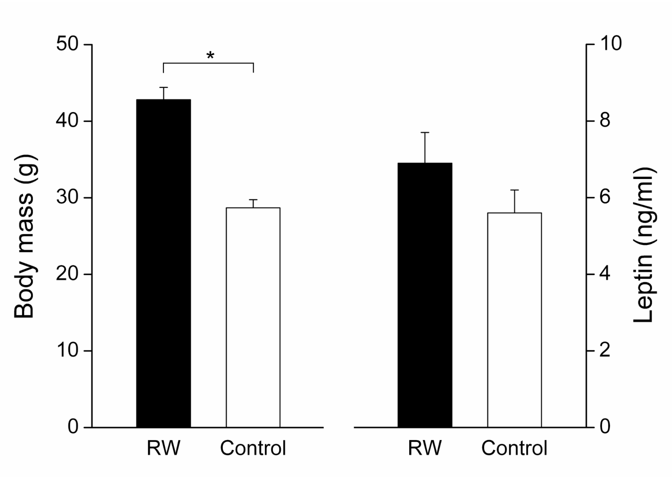

Figure 4.2. Difference in body mass (left) and plasma leptin concentration (right) between exercising (RW) and sedentary hamsters ($n = 8$, each) in December. Values are means + SEM.

Leptin concentration

At the beginning of the leptin treatment, one week before the winter solstice, body mass was significantly higher in the RW hamsters compared to control animals unlike the plasma leptin concentration (6.9 ± 0.8 vs. 5.6 ± 0.6 ng/ml; $P = 0.13$; Mann-Whitney rank sum test; Fig. 4.2).

Leptin treatment

Prior to leptin treatment sedentary hamsters were at their nadir in body mass. They were significantly lighter than the RW group (28.7 ± 1.1 vs. 42.8 ± 1.6 g; $n = 8$; $P < 0.001$; Mann-Whitney rank sum test). Only hamsters with access to a running wheel responded to subcutaneous leptin infusion with a reduction in body weight (RM ANOVA; $F = 3.3$; $P < 0.001$; Tukey; Fig. 4.3).

Body composition

Body composition was determined in February with DEXA when the body mass of the sedentary hamsters was already about 6-7 g higher in relation to the nadir in December, these animals having regained about half of the weight separating the distinctive winter phenotype from the summer hamster. Body mass, however, was still significantly different between the RW and sedentary group (46.4 ± 1.6 vs. 35.2 ± 1.6 g; $n = 8$; $P < 0.001$; RM ANOVA, see above). Despite this difference in body mass DEXA (head and bone tissue excluded) revealed no significant difference in fat mass between RW and sedentary hamsters ($P = 0.065$; Table 4.1) as opposed to the lean mass which was increased in the

RW group (Table 4.1). Bone mineral content (BMC), bone mineral density (BMD), and bone area were increased in hamsters with access to a running wheel (Table 4.2). Furthermore, on radiographs of RW hamsters the vertebral columns between head and pelvis were significantly longer (7.6%; *P* < 0.05; Mann-Whitney rank sum test) unlike the femora (*P* = 0.38) when compared to controls.

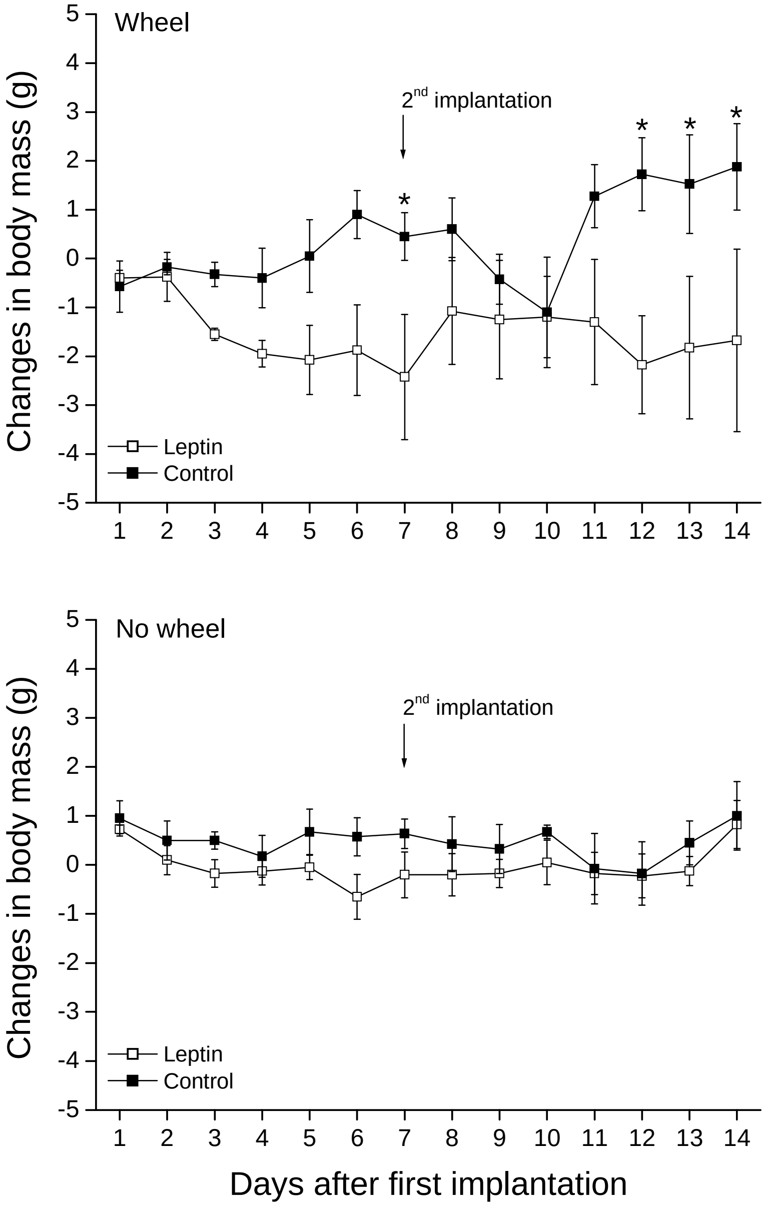

Figure 4.3. Effect of leptin treatment on body weight (December) in hamsters with and without access to a running wheel (*n* = 4/group). RM ANOVA revealed weight-reducing effects of leptin only in exercising hamsters (*P* < 0.001). Significant differences on marked (*) days (Tukey test). Values are means ± SEM.

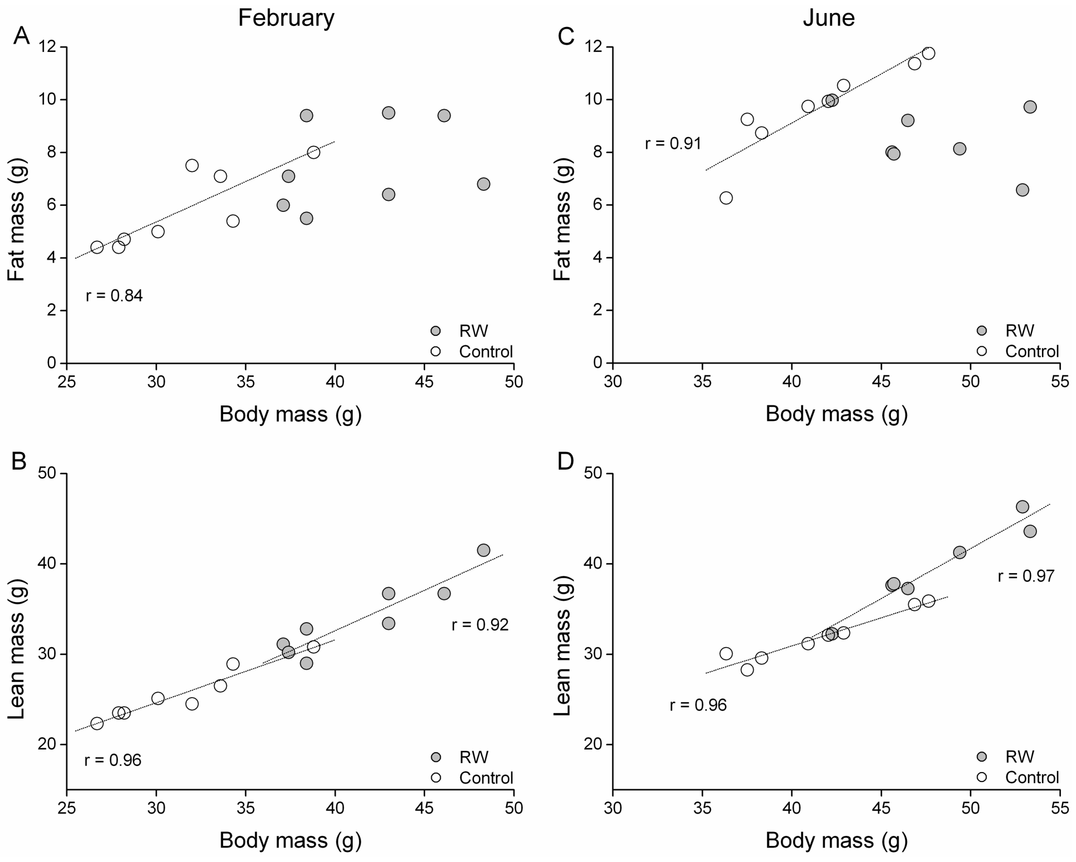

Figure 4.4. Relation between fat or lean mass and body mass in exercising and sedentary hamsters from February (left) and June. In DEXA-derived mass values (February) head and bones are excluded (see Materials and Methods). Linear regressions were highly significant (ANOVA; $P < 0.01$) except for fat mass in the RW group in both February ($P = 0.5$) and June ($P = 0.4$). Comparison (ANCOVA) of regression lines for lean mass (according to graphs) revealed a significant difference between both experimental groups in June ($F = 5.4$; $P < 0.05$).

In June body weights of the RW group ($n = 7$) were still higher in comparison to the controls ($n = 8$) (50.6 ± 1.6 vs. 43.4 ± 1.6 g; $P < 0.05$; Mann-Whitney rank sum test). This difference also applied to the heart weight (278 ± 11 vs. 234 ± 10 mg; $P < 0.05$), whereas relative heart weights (0.55 ± 0.01 vs. 0.54 ± 0.01%) were similar in both groups. Fat extraction revealed significantly increased fat mass in sedentary hamsters since February. No further increased fat content was found in the RW individuals (Table 4.1). Data of the two methods were compared directly since DEXA reliably predicts body fat content in small rodent species (Brommage 2003, Johnston et al. 2005, Nagy and Clair 2000). Although it was shown that DEXA slightly overestimates total body fat (+1.1 ± 0.13 g) in

Phodopus sungorus (Johnston et al. 2005, in agreement with our own unpublished data) the present DEXA-derived fat values were not transformed due to potential confounding effects of season, age and sex of the hamsters. Moreover, data transformation according to the published equation does not change any of the presented statistical comparisons. Dry body mass was similar in both groups (RW vs. control: 19.3 ± 0.3 vs. 19.3 ± 0.7 g). Fat-free dry mass was elevated in RW individuals compared to controls (10.8 ± 0.4 vs. 9.6 ± 0.3 g; $P < 0.05$; Mann-Whitney rank sum test).

Table 4.1. Body composition of exercising (RW) and sedentary hamsters

	Lean mass (g)		Fat mass (g)	
	Control	RW	Control	RW
February[‡]	25.6 ± 1.0	33.9 ± 1.5[a]	5.8 ± 0.5	7.5 ± 0.6
June	31.9 ± 1.0	39.5 ± 1.8[†a]	9.7 ± 0.6[b]	8.5 ± 0.5[†]

Values are means ± SEM; $n = 8$ ([†] $n = 7$); [‡] head excluded from DEXA measurement, therefore lean mass was not tested for differences between measurements; [a] significant difference compared to control group; [b] significant difference between measurements; $P < 0.01$.

Table 4.2. Bone parameters from DEXA measurement in February

	Area (cm^2)	BMC (g)	BMD (g/cm^2)
RW	10.28 ± 0.44	0.635 ± 0.031	0.062 ± 0.001
Control	8.72 ± 0.21*	0.470 ± 0.013**	0.054 ± 0.001**

Values are means ± SEM; BMC = bone mineral content; BMD = bone mineral density; $n = 8$; *$P < 0.01$, **$P < 0.001$.

Fur color

There was no difference between the groups concerning the final color stage of winter fur. Until the end of December sedentary and RW hamsters molted on average to stage 4 (median) with a range of 3-5, respectively.

Torpor

Shallow daily torpor was observed in all sedentary animals. The torpor period ranged from mid-December to the end of January. During the two-week leptin treatment with daily weight determination this winter trait was displayed at least once (median 3; range 1-6 events), respectively. In contrast, none of the RW group showed torpor.

Gonadal development

In mid-December gonadal regression in all animals was progressed so far that testes were no longer palpable (stage 0). With respect to this starting point and the defined end point (stage 3) testicular recrudescence began and was finished earlier in hamsters with access to a running wheel compared to control animals without a wheel ($n = 8$; $P < 0.05$; Mann-Whitney rank sum test; Fig. 4.5). In June, when body composition was analyzed for the second time, both RW and control group showed similar paired testis weights (875 ± 78 vs. 829 ± 26 mg).

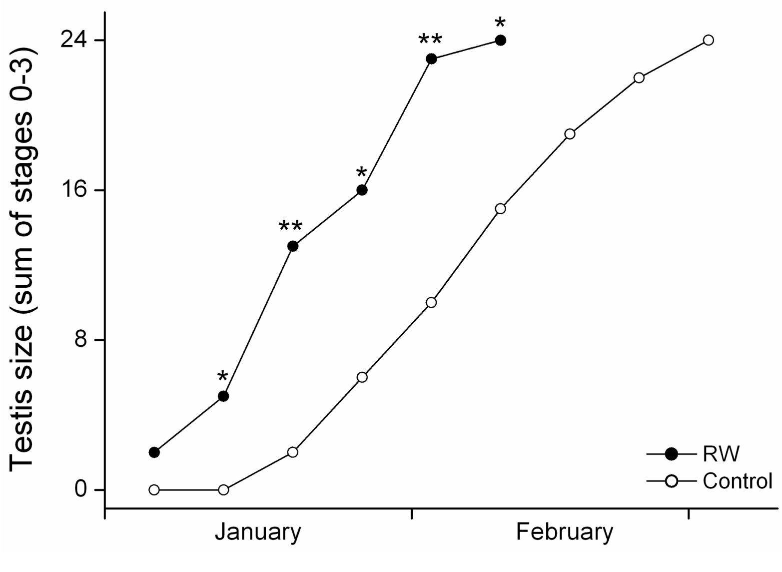

Figure 4.5. Difference in testicular development (weekly determination) between individuals with and without access to a running wheel (n = 8). In mid-December all hamsters showed stage 0 (testes not palpable). *$P < 0.05$, **$P < 0.01$.

DISCUSSION

Short-day acclimatization allows Djungarian hamsters to survive in a strongly challenging habitat. Winter traits such as reduction in body weight and daily torpor contribute to saving energy in a cold environment where food availability is low (Heldmaier and Steinlechner 1981a, b). Inhibition of both controlled hypothermia and reduction of body weight due to wheel running, as shown in the present study, would be disadvantageous under natural conditions. Therefore, one has to assume that this phenomenon is a laboratory artifact linked to the attractiveness of a running wheel. However, whether merely the activity level of a natural behavior is considerably increased or the wheel-running behavior itself is artificial remains open. Nevertheless, it might be a helpful tool for investigating the regulation of torpor as well as the seasonal body weight cycle.

In the current study depending on the onset of running wheel availability the typical weight change induced by short photoperiod was prevented or aborted by abruptly increasing body mass (Fig. 4.1). Determination of the body composition in February, when the RW individuals were on average about 11 g heavier than the sedentary controls (Fig. 4.1C), revealed that the elevation of body mass was related to an increase in lean mass (Fig. 4.4A, B). Fat mass in RW hamsters was not significantly higher than in sedentary animals (Table 4.1). Accordingly, the ratio of fat and body mass in the RW group indicates a leaner state compared to controls, because six out of eight values lie below the extrapolated regression line for sedentary hamsters (Fig. 4.4A). This difference in fatness is even more obvious in body composition data from June showing both higher fat values in sedentary hamsters and a lower proportion of lean mass (Fig. 4.4C, D). Exercising individuals almost maintained their adipose tissue mass constant since February whilst sedentary hamsters, as expected, significantly increased fat mass from winter to summer (Table 4.1). This reduced fat content in exercising hamsters was also supported by leptin analysis in December. Plasma leptin concentrations of the RW group were not significantly increased compared to the much lighter (~14 g) controls at their body weight trough (Fig. 4.2). Since the amount of leptin in blood is positively correlated with body fat in Djungarian hamsters (Klingenspor et al. 2000) one can assume that the fat mass was comparable in both experimental groups despite the significant difference in body mass, i.e. a lower fat proportion in RW hamsters. Taken together, hamsters with access to a running wheel neither showed the annual cycle in body weight nor the pronounced seasonal fluctuation in fat mass, instead body mass is increased but with decreased fatness compared to sedentary animals.

In Djungarian hamsters body weight is precisely regulated during the annual cycle being continuously adjusted to the seasonally appropriate weight (Morgan and Mercer 2001, Steinlechner et al. 1983). Thus, at first view exercise-induced elevated body weight during winter could simply reflect the typical summer level. RW animals, however, differed clearly in plasma leptin from equiponderate summer hamsters which show considerably higher concentrations (Freeman et al. 2004b) due to a high fat content (Wade and Bartness 1984). This indirect evidence for a summer-inadequate fat content in RW animals, underlined by later direct measurements, is contrary to the hypothesis of a running-induced body mass in winter that reflects the summer state. Fat mass of exercising individuals, however, was more variable relative to body weight both in February and June (Fig. 4.4A, C). This lack of a definite correlation between fat and body mass indicates a varying effect of wheel running presumably due to a different running quantity and/or evoked by individually unequal physiological responses.

Constantly low body fat of exercising hamsters could simply be a result of increased energy expenditure due to extensive locomotion. Consistent with this interpretation the chemical carcass analysis showed a significantly higher proportion of body water in RW hamsters in June maybe due to increased glycogen storage in liver and muscles. Moreover, BMD and BMC were significantly increased in RW hamsters in February, probably reflecting a physiological/anatomical adaptation to the physical strain. However, a parameter for endurance performance, the relative heart weight, was not elevated in exercising animals compared to controls at the end of the experiment.

Controversial to the present results and our similar findings under LD 16:8 (unpublished), in a former study *Phodopus sungorus* showed no significant body weight increase when given access to a running wheel (~32 cm diameter) (Bartness and Wade 1985). This lack of any weight-inducing effect is possibly linked to the lower amount of activity. After two weeks, revolutions per day (rev./d) declined and remained below 6,000 approximating a distance covered of less than 6 km. The present investigation revealed about 50% higher mean values at least during the first three months of access to a running wheel (experiment 1: ~30,000 rev./d, experiment 2: ~20,000 rev./d, mean of the first 8 weeks, respectively; experiment 3: ~20,000 rev./d, mean of 2 weeks in February and March). Simple comparison of the covered distances, however, has to be considered carefully because of unequal wheel sizes requiring different postures and torques. Indeed, in Syrian hamsters it has been demonstrated that growth is only accelerated by more than 15,000 rev./d on an activity disc (Borer and Kaplan 1977) indicating as precondition a minimum exercise level that is considerably higher than general activity.

Since the higher body mass of RW hamsters in the present study was not caused by fattening, stimulated growth could be a consequence of wheel running. Measurement of bones in DEXA radiographs taken in February revealed significantly lengthened vertebral columns (~7%) in exercising hamsters which is well in accordance with the results for Syrian hamsters (Borer and Kuhns 1977). Furthermore, significantly increased bone area in the RW group strongly supports the growth hypothesis. SD-induced decrease in body mass should be associated with a discontinuation of growth, which could well be prevented in hamsters with access to a running wheel. Supporting this interpretation, RW animals were still significantly heavier with increased fat-free dry mass when controls reached the summer plateau.

Annual cycles of adiposity and body mass have been associated with the adipose-derived hormone leptin known for its anorectic effect (for review, see Ahima and Flier 2000). A reduced leptin sensitivity in long-day animals compared to short day-acclimatized individuals was shown for Djungarian hamsters (Atcha et al. 2000, Klingenspor et al. 2000) and field voles (*Microtus agrestis,* Król et al. 2006) suggested to be regulated mainly by photoperiod (Rousseau et al. 2002). In the current study, however, sedentary SD hamsters showed no reduction in body mass during leptin treatment as opposed to the RW animals with higher initial body weights. In preceding investigations leptin effects were tested already after 8 weeks of SD when individuals were in the middle of transition. Absence of a reaction to leptin in this study could be due to the different state of acclimatization. Prior to leptin treatment the sedentary hamsters had reached the nadir in body weight, which can be expected to be strongly defended. Another explanation for the lack of leptin sensitivity is provided by results concerning the underlying mechanisms. The suppressor of cytokine signaling 3 (SOCS3) has been shown to play a critical role in leptin sensitivity in mice (Howard et al. 2004), field voles (Król et al. 2007) and Djungarian hamsters (43). This protein inhibits signal transduction distal of the leptin receptor, and an increase of arcuate nucleus SOCS3 gene expression was shown to precede the long photoperiod-induced increase of body weight in Djungarian hamsters by approximately 3 weeks (Tups et al. 2006). Hence, in the present study sedentary SD hamsters failed to respond to leptin administration possibly due to an already raised SOCS3 level causing leptin resistance. Whether the leptin response in RW hamsters was related to the latter pathway, e.g. a low SOCS3 level, or merely to an increased potential for weight loss simply due to the higher body mass, or both, remains speculative.

For Syrian hamsters exercise-induced increase in body weight has been reported as well as effects on testis development. Although testicular regression is incomplete in Syrian hamsters with access to a running wheel (Gibbs and Petterborg 1986) neural integration of the shortened photoperiod is not prevented (Menet et al. 2005). Lack of perception of changing day length in exercising individuals can be excluded for *Phodopus sungorus* as well because of the present findings of complete gonadal regression and the change to winter fur. Since the course of testes involution has not been observed it is not known whether the SD-induced testicular development on the whole was shifted forwards or compressed. In any case, the recrudescence was advanced in Djungarian hamsters with access to a running wheel, indicating either an advanced termination of the winter state, i.e. refractoriness, or a testes-stimulating effect prevailing when suppressing signals become weaker or disappear. In the latter case the testis regression should be decelerated in exercising hamsters. In this regard, it cannot be ruled out that a different pattern of entrainment and thus an altered photoresponsiveness is responsible for some of the effects in RW hamsters, as has been found in selectively bred photoperiodic nonresponsive hamsters that exhibited adjustments to SD only when given access to a RW (Freeman and Goldman 1997). A pre-pineal influence of wheel running on the entrainment would explain the lack of weight-increasing effects in the study of Bartness and Wade (1985) where SD was mimicked by melatonin injections. More recent findings indicate that the thalamic IGL is involved in the photoperiodic responsiveness and the activity feedback mechanism (Freeman et al. 2004a, 2006).

Another typical SD trait, daily torpor, was not observed in RW individuals, confirming the results of Thomas et al. (Thomas et al. 1993). The absence of torpor, however, was probably not due to an elevated leptin level which was previously discussed to prevent hypothermia (Freeman et al. 2004b). Firstly, there was no obvious difference in torpor frequency between the leptin treated sedentary group and the untreated sedentary animals, thus corresponding to the results of Schmidt et al. (Schmidt et al. 1997), and secondly, in December, the plasma leptin concentration in the exercising hamsters with high body weights was only slightly increased (n. s.) compared to sedentary animals. In this regard in *Phodopus sungorus* a negative correlation was found between the individual number of torpor episodes and the general intensity of nocturnal locomotor activity (Ruf et al. 1991, 1993). High torpor frequency was assumed to facilitate a constant low activity level, probably reflecting reduced foraging, which in combination yields an effective energy saving strategy. In contrast, wheel running possibly shifts the hamsters to the other end of the behavioral range characterized by a very low torpor frequency and a higher general activity level. Furthermore, physiological parameters related to hypothermia such as metabolism and body temperature which are influenced by wheel-running activity may be responsible for inhibition of torpor.

In conclusion, wheel running strongly affected the typical seasonal acclimatization of Djungarian hamsters. As a consequence testes recrudescence was advanced and the reduction in body mass as well as daily torpor were inhibited. Elevation in body mass was due to significant increases in lean mass, and the fat content was reduced compared to sedentary hamsters. This alteration of body composition was accompanied by considerably increased mineral content and density in bones reflecting the diversity of physiological responses due to wheel-running activity.

5

Acute and lasting effects of wheel running on body mass and torpor

ABSTRACT

Djungarian hamsters (*Phodopus sungorus*) were exposed to artificial short days (SD) either with access to a running wheel (RW) or without. Within six weeks RW hamsters had increased their body mass by about 5 g, whereas sedentary controls showed the typical body mass reduction. Estimation of paired testis weights indicated a decelerated testis regression in RW hamsters. Subsequent locking of RWs (nine weeks) led to a decline in body mass of RW animals in parallel to controls. Daily torpor was almost completely missing in hamsters with initially unlocked wheels. During the final phase RWs were again unlocked (three weeks) and body mass of exercising hamsters increased again, while controls reached the nadir in body mass. Body composition was affected insofar as the relative heart weight was significantly lower in RW animals compared to SD controls. In comparison to equiponderate long day (LD) controls the relative liver weight of RW hamsters was significantly increased unlike the relative heart weight. However, the latter tended to be higher than in sedentary LD hamsters. A growth-stimulating effect of wheel running was proven by elongated femora in exercising SD hamsters compared to SD controls and, furthermore, suggested also by exercise-induced elevation of relative food intake and body mass under LD conditions, indicating a growth-promoting effect of wheel running independent from the photoperiod. Inhibition of SD-mediated body mass reduction due to running exercise and the potential to lose body mass in artificial SD when wheel running is prevented was confirmed under natural lighting conditions with natural ambient temperature.

INTRODUCTION

Wheel-running activity was previously shown (chapter 4) to affect the seasonal acclimatisation in Djungarian hamsters (*Phodopus sungorus*). Most strikingly, the typical short day-mediated body mass reduction was either inhibited or discontinued depending on seasonal phase when hamsters were initially allowed free access to a running wheel (RW). In fact, when hamsters obtained RW access during the transition from a heavy summer hamster to the considerably lighter winter phenotype, body mass showed a steep rise due to wheel running, indicating the strong impact of pronounced exercise on the hamster's physiology. Not only was the annual cycle of body mass affected but also that of testis size, underlying seasonal reproduction. In contrast to the body mass reduction the concomitant testis regression was obviously not prevented, which was proven by non-palpable testes in RW hamsters in winter. Testicular recrudescence usually occurs spontaneously after prolonged SD exposure when the animals have become refractory to the 'inhibitory' photoperiod (Hoffmann 1973, Steinlechner et al. 1983). This was also the case in RW hamsters but re-growth of the testes was advanced when compared to controls having no access to a wheel.

In contrast to the findings for the body mass cycle and testicular development, a further winter trait, the change in fur colouration, seemed to be unaffected by wheel running, confirming earlier results (Thomas et al. 1993). This conclusion, however, is merely based on the final colour stage reached in winter (chapter 4) and the overall impression in artificial SD (data not shown; Thomas et al. 1993). Hence, by means of existing data it cannot be ruled out that the temporal course of the change to white winter fur is in fact altered due to wheel running, although the final state is not distinguishable from the level of colouration in controls. Therefore, one aim of the present study was to clarify this point. For comparison of the colour change progression, two different kinds of determination were chosen. Since in the previous study only the testicular recrudescence was checked, here the initial change of the testis cycle, namely the testis involution, was the focal point in order to complete the overall picture. Furthermore, the sustainability of the wheel running-induced increase of body mass was verified by simply locking the wheels after a preceding period of free RW access. Moreover, investigations were extended to LD conditions eliminating the aspect of seasonality. All in all, the study was carried out to further characterise the complex phenomenon of wheel running affecting physiology and morphology in Djungarian hamsters. This may provide the basis for new insights into the regulation of body weight in general and as a seasonal trait.

MATERIALS AND METHODS

Male Djungarian hamsters (*Phodopus sungorus*) were bred and raised outdoors under natural lighting conditions (~52° N latitude) with natural ambient temperatures (T_a). After weaning, the animals were kept singly in polycarbonate cages (Makrolon type II) with tap water and food (hamster breeding diet, Altromin 7014) available ad libitum, supplemented weekly by a slice of apple.

Experiment 1. On August 3rd 20 hamsters (7-11 weeks old) were transferred into a temperature-controlled chamber (21 ± 1°C) with a light-dark cycle of 16 h of light and 8 h of darkness (lights on from 5:00 h to 21:00 h; CET) to maintain the animals' physiological and morphological summer state. Seven weeks later the lighting conditions were changed from long-day (LD) to short-day (SD) conditions (lights on from 8:00 h to 16:00 h), while T_a remained unaltered. With the beginning of exposure to a short photoperiod one group of hamsters gained access to a running wheel (RW; ~14 cm inner diameter) inside their standard cages. Only in the beginning was the use of the RWs checked with the help of video surveillance. Usually hamsters that start once to use their wheel will do so again every day. Both weight-matched groups, i.e. RW hamsters ($n = 11$, see below) and sedentary controls ($n = 8$), were weighed twice a week to the nearest 0.1 g. After six weeks in artificial SD the RWs were locked for nine weeks and then released again until the end of the experiment after 18 weeks of SD exposure. The data of one RW animal were excluded from all analyses because of an abrupt and severe loss in body weight followed by only slow recovery, indicating temporary physical impairment. Testis size was measured externally with a calliper under slight isoflurane anaesthesia one day before the change from LD to SD conditions and every other week in SD until precise determination became impossible due to pulpiness of the testes. Paired testis weight (PTW) was estimated using an equation from Watson-Whitmyre and Stetson (1985). Changes in fur colouration from greyish-brown to white winter fur due to the shortened photoperiod were determined by two different methods on the basis of pictures taken at two week intervals while the hamsters were anaesthetised for testis palpation. Pictures of the dorsal side of outstretched hamsters lying on a white paper (with a black area) were adjusted for brightness in Photoshop 6.0. With another software (ImageJ) the grey value of the hamsters' back and sides was determined as an objective measure. A large as possible area was selected using an ellipse not exceeding the animals' outline. Besides, a technical assistant unaware of the experiment determined the fur colour index according to the six stages defined by Figala et al. (1973).

With the beginning of the torpor season in SD the hamsters were checked for exhibiting controlled hypothermia during the photophase (~4-5 h after lights on) of 3-6 days (median 5) within each week. For presentation of the results, data from each week in SD were merged. The number of days (per week) with observation multiplied with the number of RW or control individuals adds up to the respective maximum of torpor events (100%) that could have been found if all animals had shown torpor when checked for it.

After 18 weeks of SD exposure animals (~8 months of age) were culled with carbon dioxide for organ dissection. Organs were weighed to the nearest 1 mg (RC 210 D, Sartorius Inc., Goettingen, Germany). The length of the hamsters' excised left femur was determined with a calliper. The same procedure was carried out with an additional ten male hamsters (~10.5 months old) representing LD control hamsters that had regained weight up to the summer level after their previous SD acclimatisation. For that the young animals were transferred (at the summer solstice) from natural lighting conditions and natural T_a to comparable artificial LD conditions (16.8 h light : 7.2 h darkness; 6 weeks) and constant T_a (20 ± 1°C) followed by SD exposure (28 weeks). At the end of the expanded SD exposure all individuals were not only photorefractory (longest) but had already reached on average the body weight they had had before SD acclimatisation (see suppl. Fig. S.4 for body weight record). In order to ensure that the hamsters could serve as control summer hamsters they were finally exposed to LD conditions again for about five weeks before they were sacrificed (Fig. S.4). The sample size for the paired kidney weight was reduced to $n = 9$ because of a pathologically increased kidney size in one LD control animal.

Food consumption was determined for the three different groups shortly before they were killed for weighing the organs. The food was dried (4 days; 60°C) and weighed to the nearest 0.1 g before and after it was fed to the hamsters (4-6 days). Body mass values of each hamster from just before, during and at the end of the food intake experiment were averaged for calculation of relative food consumption.

Experiment 2. Body weight of eight hamsters kept outdoors under natural lighting conditions and natural T_a was checked for about 20 months including two consecutive winters. At the summer solstice one of the weight-matched groups (7 weeks of age; *n* = 4, each) obtained free access to RWs, whereas their siblings remained without RW access. After one year the latter condition was inverted.

Individuals of two more experimental groups were born four months later at the end of the natural breeding season. One group (*n* = 5) gained access to RWs on October 11th at about six weeks of age. The other group (*n* = 4-7; age-matched) served as control group without wheels.

Experiment 3. Fifteen male hamsters (11-13 months old) were moved on April 11th from outdoors with natural lighting conditions and natural T_a into a chamber with artificial LD conditions (lights on from 6:00 h to 22:00 h; CEST) and constant T_a (22 ± 1°C). After 25 days in LD all animals were transferred from standard cages (Makrolon, type II) to special cages (~24.5 × 15 × 15 cm; volume ~5.5 l) suitable for indirect calorimetry and in part equipped with a RW (inner diameter 14 cm). Furthermore, a plastic box with a lid and a removable metallic rack inside was fixed to the long side of the cages for measuring food intake. Both wheel-running activity and general activity (passive infrared (IR) detector; Conrad Electronic SE, Germany) were registered continuously and stored every six minutes on a personal computer. Oxygen (O_2) consumption and production of carbon dioxide (CO_2) were determined (6-min intervals) in an open system with the help of an analyser (Multor 610, Maihak) measuring both gas concentrations of the dried air (flow: ~35 l/h) coming from the animal's cage (for details, see Dernbach 2002). Due to limited capacity (six channels) O_2 and CO_2 could be measured for five hamsters only (one reference channel) at a time, i.e. measures were carried out consecutively with three mixed groups of five individuals for four days, respectively. Food consumption was determined continuously throughout the experiment with weighing intervals of one day (first 4 weeks), two days (following 10 weeks) or four days (last 3 weeks). The racks were weighed before and after filling with food pellets, and remaining crumbs were collected from the cages and weighed for calculating the food intake per day. In order not to falsify measures of O_2 and CO_2, cages were not opened for collection of remaining food until the end of the 4-day records. From the outset the entire food required was kept in the temperature- and humidity-controlled chamber in order to have pellets with comparable water content throughout the experiment. Values are given as mean and SEM if not stated otherwise.

RESULTS

Experiment 1. The body weight course of both experimental groups was similar until they were transferred from LD to SD. In addition to the change in photoperiod one group was provided with RWs and increased body mass by 4.8 ± 1.3 g within the six weeks of free RW access in SD (Fig. 5.1), whilst sedentary controls responded to the 'inhibitory' photoperiod with a reduction of body mass (–3.6 ± 1.1 g) as expected. Divergence of weight development was significant already after 25 days in SD (RM ANOVA; $F = 15.8$; $P < 0.001$; Tukey), and this difference in body mass remained significant until the end of the experiment. During the nine weeks of blocked RWs (week 7-15 of SD exposure) not only body mass of sedentary controls continuously decreased but also that of RW individuals (Fig. 5.1; suppl. Fig. S.3). Loss of body mass in control animals was 3.0 ± 0.5 g (9.0 ± 1.6%) compared to 6.0 ± 1.3 g (13.8 ± 3.0%) in RW hamsters within the blockage phase. This difference was not significant (see also suppl. Fig. S.3), indicating a comparable rate of weight loss in the RW group.

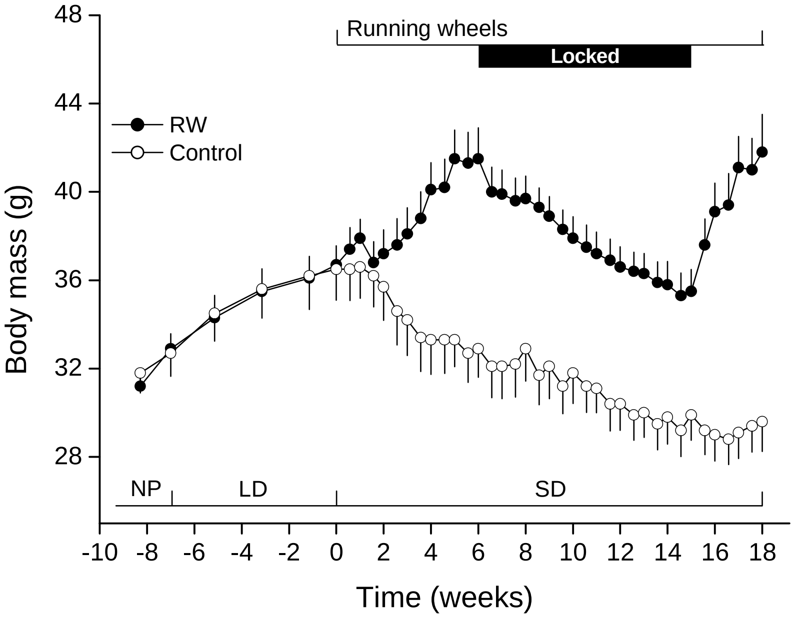

Figure 5.1. Mean body mass (SEM) of hamsters with access to running wheels ($n = 11$; RW temporarily blocked) after transfer from long days (LD) to short days (SD), and of sedentary controls ($n = 8$) without access to a wheel. NP = natural photoperiod.

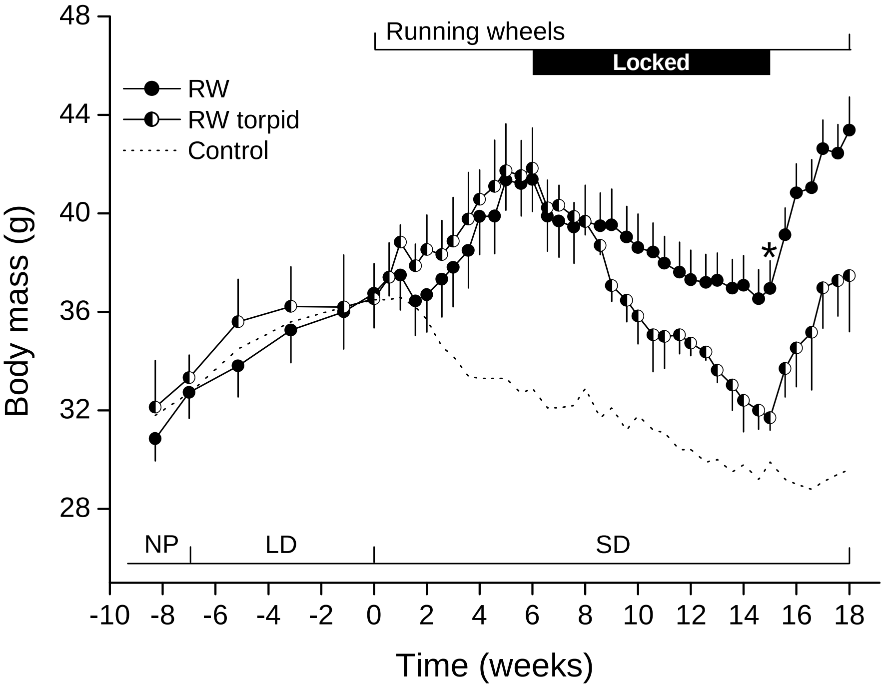

Figure 5.2. Body mass (and SEM) of RW hamsters that displayed torpor at the end of the phase of locked wheels (*n* = 3) in comparison to the course of body mass in hamsters where no torpor event was observed (*n* = 8). The dotted line shows the course in body weight of controls (*n* = 8) that invariably displayed torpor. The group of RW hamsters that showed torpor was lighter than the remaining RW animals at the end of the decline (see asterisk; $P < 0.05$; Mann-Whitney rank sum test) but not different from the controls. Only the last time point prior to the release of running wheels was considered for comparison of body weights.

However, if the RW hamsters that displayed torpor during the phase of locked wheels were compared to those in which torpor had never been observed, the former group was significantly lighter at the end of the body mass decline, and not significantly heavier than the sedentary controls (Fig. 5.2). The change in body mass of RW hamsters that showed shallow torpor was –10.1 ± 2.3 g (−24.1 ± 3.9%) vs. –4.4 ± 1.4 g (−9.9 ± 3.0%) in animals in which no torpor event was observed during the blockage phase. Until their nadir in body weight the sedentary controls lost 7.8 ± 1.2 g (20.8 ± 3.0%) of their body mass with respect to the highest mean value prior to the SD-induced decline. Within the second period of access to unlocked wheels (3 weeks; Fig. 5.1) the rate of gaining body mass (0.297 ± 0.05 g/d) was significantly increased compared to the first phase (0.115 ± 0.03 g/d; $P < 0.01$; Mann-Whitney rank sum test).

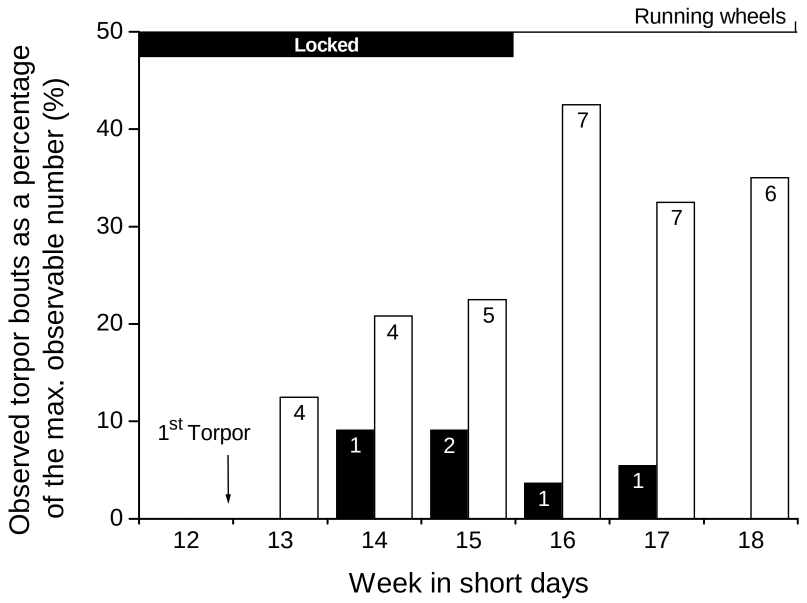

Figure 5.3. Comparison of torpor occurrence between hamsters with access to a running wheel (black bars; *n* = 11) and without (*n* = 8). For each week of SD exposure observed torpor bouts are given as percentage of the calculative maximum for the group, i.e. the number of days per week when the animals were checked, multiplied with the number of animals. The number of individuals contributing to the shown results is given inside the bars.

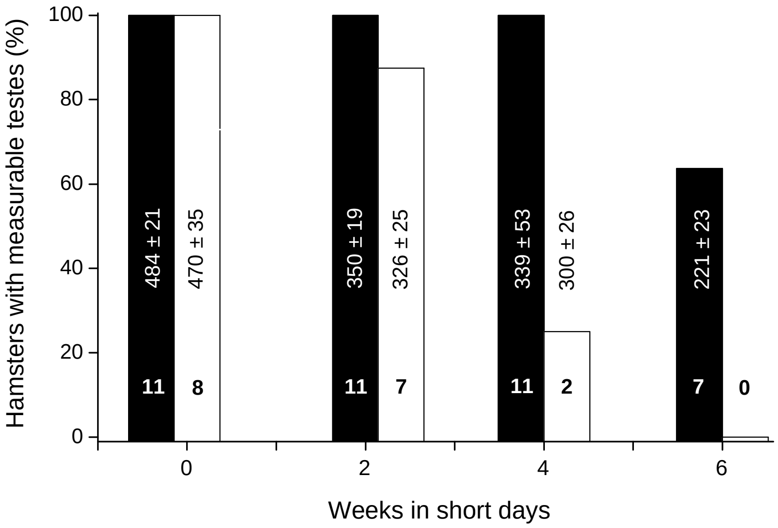

Figure 5.4. Percentage of RW hamsters (black bars) and controls with testes that were measurable with a calliper just before and during SD exposure. Testes of remaining animals already became too soft for measurement. The number of individuals and their mean estimated paired testis weight in mg (± SEM) are given inside the corresponding bar.

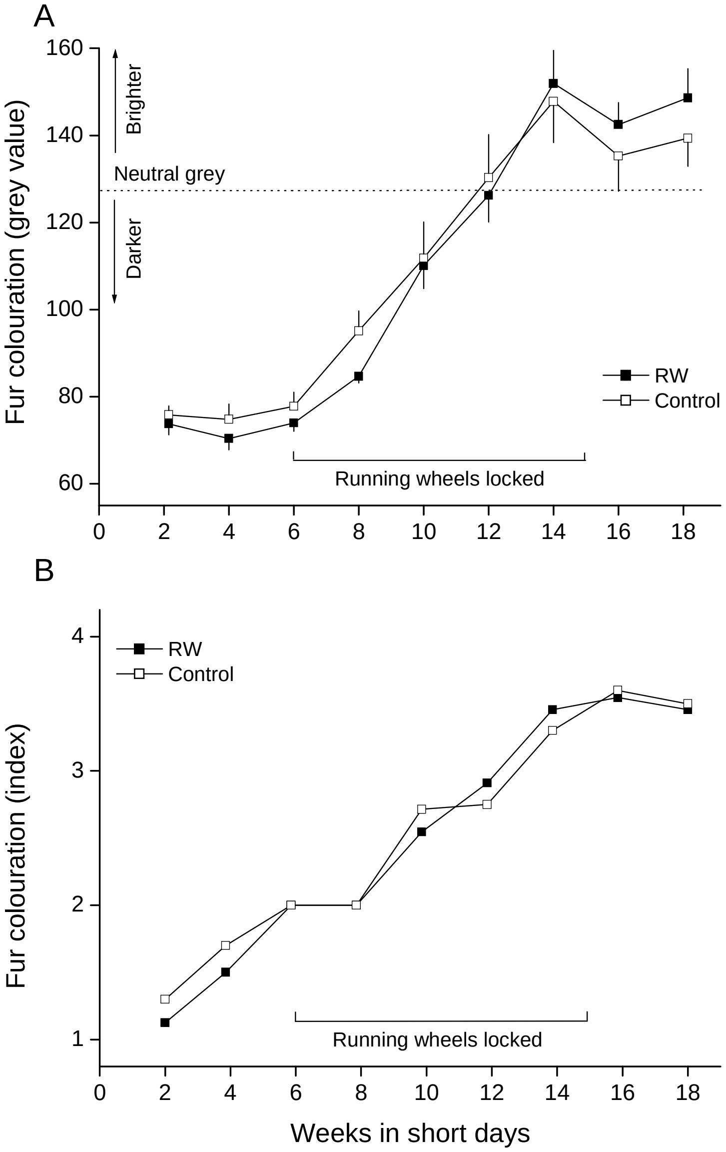

Figure 5.5. Change in fur colouration during SD exposure. A: Mean grey value (0 = black; 255 = white; 127.5 = neutral grey) and SEM for hamsters with (*n* = 11) and without access (*n* = 8) to a running wheel (temporarily locked). B: Corresponding mean fur colour indices (1 = greyish brown summer fur; 6 = white winter fur) determined by using the same pictures as for A.

The first torpor event was observed in a control animal on the last day of the 12th week of SD exposure, which was the beginning of observation (Fig. 5.3). Eight days later torpor occurred for the first time within the RW group (but wheels locked!). During the torpor season all control animals showed controlled hypothermia in contrast to only three RW individuals. The frequency of torpor in controls increased with the duration of exposure to

SD and reached a peak in week 16 with seven out of eight animals displaying controlled hypothermia. After releasing the locked RWs one RW hamster continued to show torpor. Video surveillance revealed that this animal did not initially use the unlocked wheel. After resuming wheel-running activity, this individual stopped displaying torpor similar to all other exercising hamsters. Effectively occurred torpor bouts as a percentage of the calculative maximum are shown in Fig. 5.3. Comparison of the change in fur colouration between (temporarily) exercising hamsters and sedentary controls revealed no difference in the course of turning to a whitish coat colour (Fig. 5.5). One day before the beginning of SD exposure the estimated PTW was similar for both groups (Fig. 5.4). Subsequently, the percentage of hamsters with still measurable testes decreased faster in the control group compared to RW animals in SD.

Table 5.1. Different body parameters and food consumption of hamsters kept either under short days (with or without a wheel) or under long days (without a wheel).

	SD		LD	
	Control (*n* = 8)	RW (*n* = 11)	Control (*n* = 10)	Tukey test *P* value
Body mass (g)	29.4 ± 3.4[a]	41.3 ± 5.0	41.6 ± 5.2	< 0.001
Food intake (g/d)	2.92 ± 0.37[a]	4.75 ± 0.78	4.20 ± 0.54	< 0.001
(g/d · g BM)	0.10 ± 0.02	0.115 ± 0.01	0.103 ± 0.02	
IBAT (mg)	157 ± 45	221 ± 74	194 ± 70	
(%)	0.53 ± 0.10	0.52 ± 0.13	0.46 ± 0.13	
Liver (mg)	1311 ± 204	2015 ± 219	1560 ± 202	< 0.05
(%)	4.43 ± 0.38	4.82 ± 0.35	3.76 ± 0.45[a]	< 0.01
Kidney (mg)	363 ± 61[a]	506 ± 76	539 ± 67[†]	< 0.001
(%)	1.23 ± 0.16	1.21 ± 0.14	1.29 ± 0.14[†]	
Heart (mg)	204 ± 27[b]	234 ± 17	212 ± 21	< 0.05
(%)	0.69 ± 0.05[a]	0.56 ± 0.04	0.51 ± 0.06	< 0.001
Paired testis (mg)	45 ± 11	287 ± 145	821 ± 86	< 0.001
(%)	0.15 ± 0.02	0.67 ± 0.30	1.99 ± 0.24	< 0.001
Femur (mm)	17.6 ± 0.4	18.4 ± 0.7	19.2 ± 0.4	< 0.05

Values are means ± SD; IBAT = interscapular brown adipose tissue; % = percentage of body mass; significant differences are indicated by [a] (compared to other groups) or [b] (compared to RW) or only by *P* value (all groups are different from each other); [†] *n* = 9.

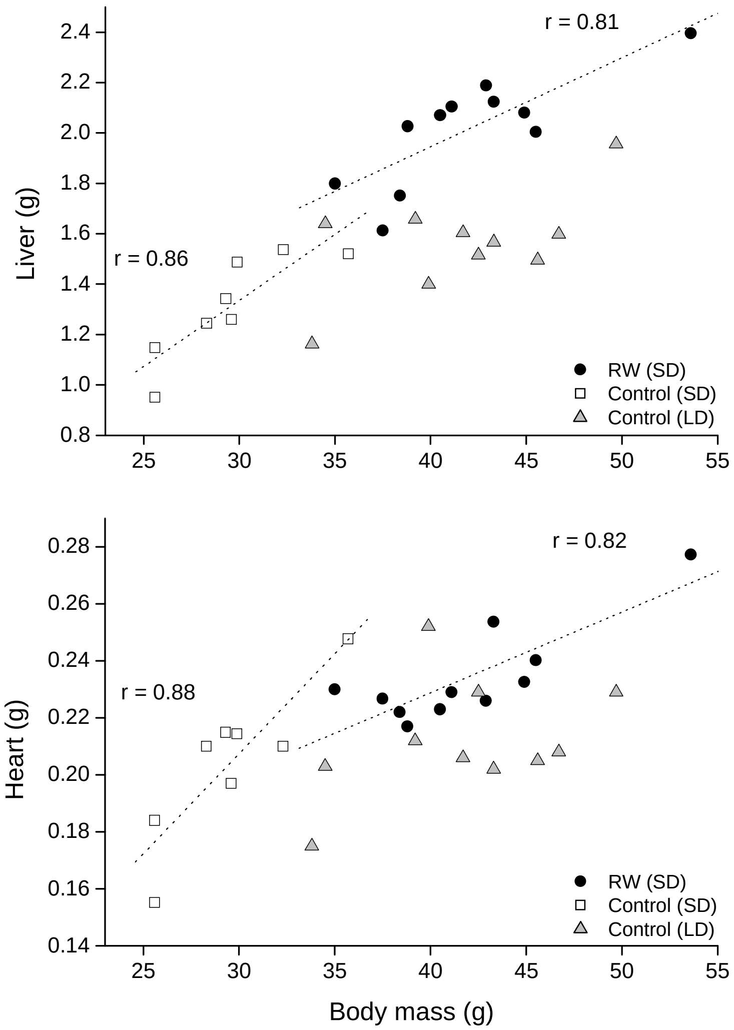

Figure 5.6. Relation between liver or heart weight and body mass in exercising (RW) and sedentary hamsters after prolonged SD exposure, or in LD controls (also without wheel). For correlations with significant Pearson correlation coefficient ($P < 0.05$) regression lines are depicted (ANOVA; $P < 0.01$).

After 18 weeks of SD exposure the animals were sacrificed for determining different organ weights. The same procedure was carried out with an additional group of LD-acclimatised individuals of a similar age. Measurements revealed a significantly increased relative liver weight in both RW hamsters and controls after the 18-week SD exposure compared to LD controls (Fig. 5.6), which contrasts to the relative paired kidney weight which was similar for all groups (Table 5.1). The relative heart weight was significantly elevated in SD controls compared to both remaining groups (Fig. 5.6). Femur lengths were different between all groups with the lowest values for SD controls and the highest values in LD controls. Unlike the daily food intake per gramme body mass, which was not significantly different between the three groups, absolute daily amounts were higher in RW hamsters and LD controls without a significant difference between both groups (Table 5.1).

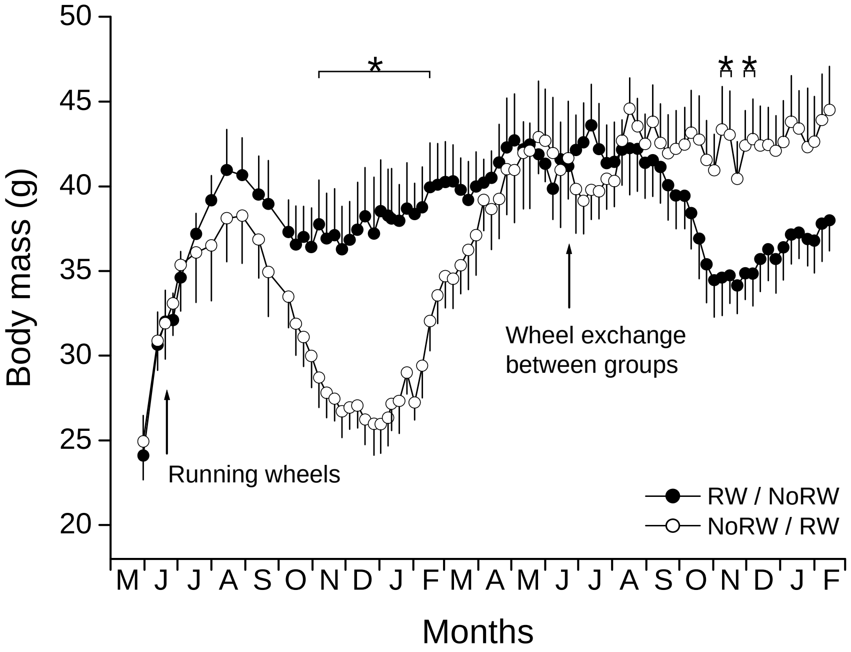

Figure 5.7. Mean body mass (SEM) of hamsters over ~20 months. One group was allowed free access to a running wheel during the first year (closed circles; *n* = 4), whereas the second group had RW access only during the last eight months, i.e. after the wheel exchange (open circles; *n* = 4). Significant differences in body mass between groups are indicated (RM ANOVA; *F* = 15.5; *P* < 0.001; Tukey).

Experiment 2. Access to a RW prevented the typical seasonal reduction in body mass exhibited by the control group (–31.3 ± 6.0%; Fig. 5.7). However, the loss in body mass was not entirely inhibited in RW individuals (*P* < 0.01; paired t-test) unlike the results in chapter 4 (Fig. 4.1B). In hamsters born after the summer solstice and without RW access, SD seemed to decelerate the increase in body mass when compared to the juvenile growth phase of the earlier born control animals in June (Fig. 5.8). Despite the age difference of both sedentary control groups their course in body weight was almost congruent after the younger animals had reached the body weight of the older winter-acclimatised hamsters. In contrast, the younger RW hamsters increased body mass beyond the appropriate weight within the typical acclimatisation exhibited by sedentary individuals. A comparison of body weight changes (first value = reference) between both groups was made instead of using absolute values in order to account for the weight difference (2.35 g; n.s.; Fig. 5.8) at the beginning.

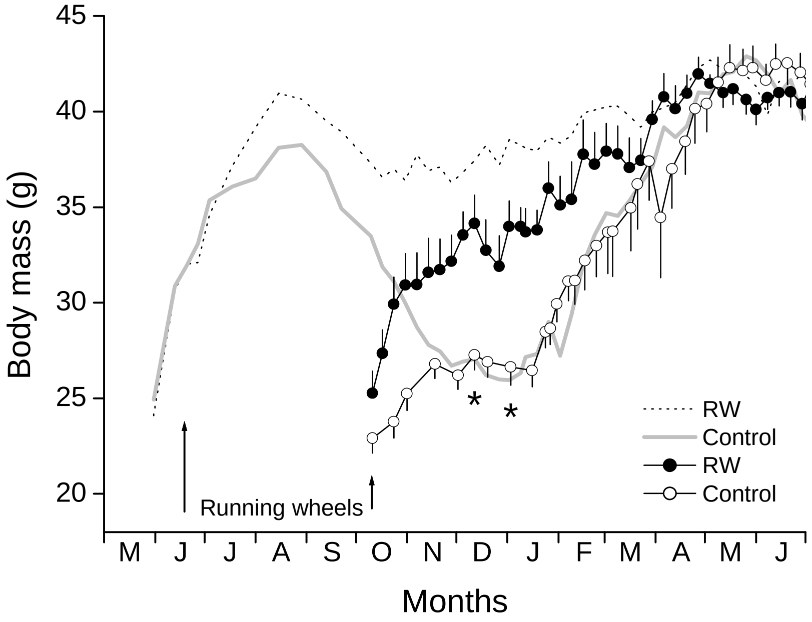

Figure 5.8. Course of body mass (mean and SEM) in hamsters with (*n* = 5) and without running wheel access (*n* = 4-7) that were born late in the breeding season. Time points with significant differences in the change of body mass (first body mass value = 0; see Results) are indicated by asterisks. Additionally, data of hamsters that were born earlier in the year are shown for comparison.

Furthermore, since both groups were often weighed on different days, values were only considered when determined on two consecutive days for both groups. Comparison of the weight change revealed significantly elevated values for RW hamsters, indicating an accelerated increase in body mass (RM ANOVA; $F = 7.6$; $P < 0.001$; Tukey).

In the following summer the four different groups reached a comparable body weight level. After the wheel exchange between both older groups at the second summer solstice of the experiment the new/current RW group (formerly without wheel) did not show the seasonal weight decrease again. However, the new/current control group that lost RW access responded to decreasing day length and significantly reduced body mass (–18.9 ± 1.3%; RM ANOVA; *F* = 15.5, *P* < 0.001; Tukey; Fig. 5.7).

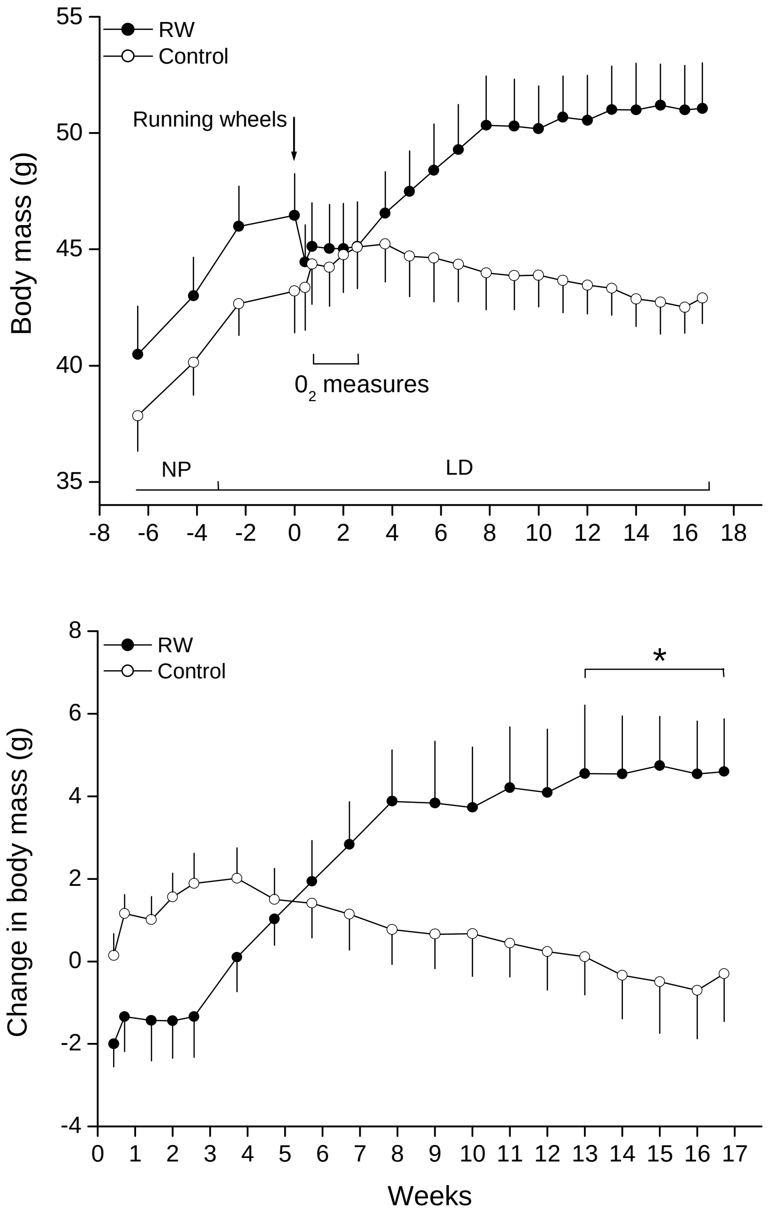

Figure 5.9. Absolute body mass (A) and changes in body mass (B) of hamsters with a wheel (*n* = 8) and without running wheel access (*n* = 7). Significant differences in the change of body mass between both groups are indicated by asterisk and line (RM ANOVA; *F* = 10.1; *P* < 0.001; Tukey). Values are given as mean and SEM.

Experiment 3. Body weight changes in RW hamsters were different from those of control animals without RW access (Fig. 5.9). From the 13th week the increase in body mass of exercising individuals (+4.6 ± 1.7 g) was significant compared to the controls that exhibited a body mass not different from the initial value (+0.1 ± 0.9 g).

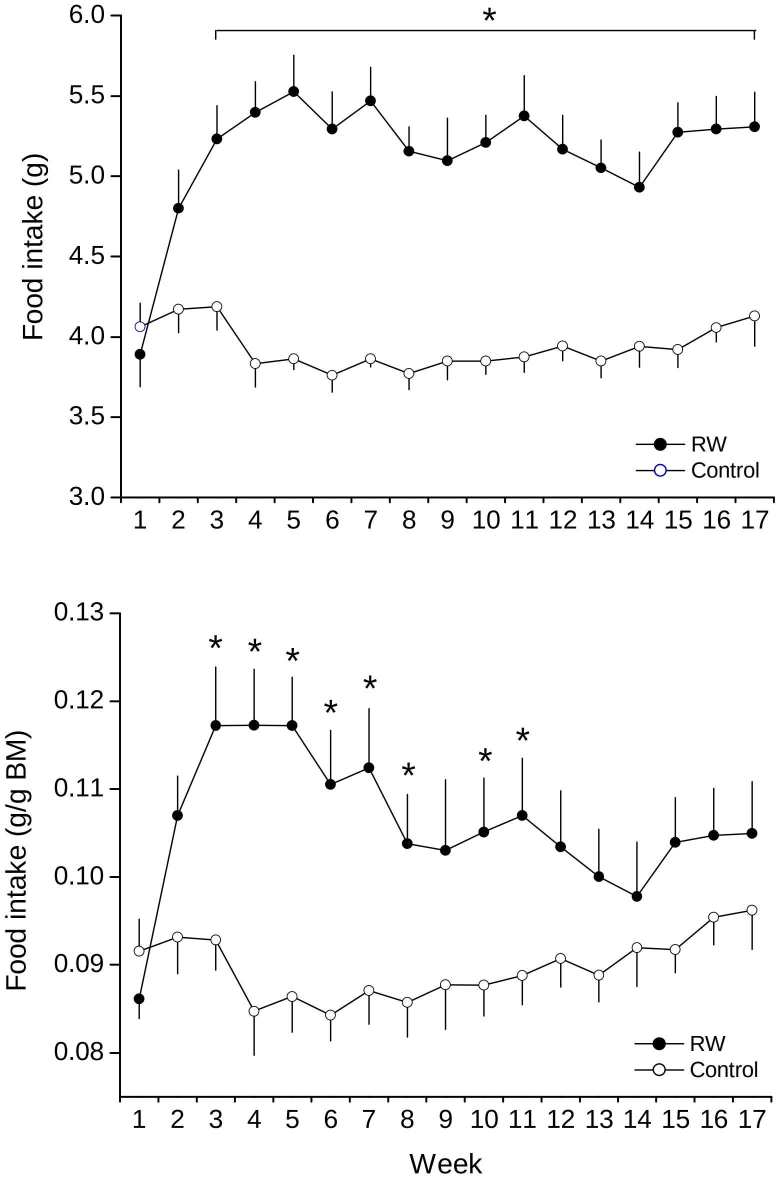

Figure 5.10. Daily food consumption (weekly mean and SEM) of hamsters either provided with a running wheel ($n = 8$) or housed without one ($n = 7$). A: Total daily food intake and significant differences between groups (RM ANOVA; $F = 5.7$; $P < 0.001$; Tukey). B: Relative daily food consumption with indicated (asterisks) significant differences (RM ANOVA; $F = 4.7$; $P < 0.001$; Tukey).

Daily Food intake also increased significantly within three weeks in RW hamsters (Fig. 5.10). Unlike the total food consumption relative intake decreased with increasing body mass during the experiment to values not significantly different from those of control animals (Fig. 5.10). In the course of the experiment also wheel-running activity decreased, namely from the maximum value of ~25,000 revolutions per day in the third week to ~10,000 (Fig. 5.11).

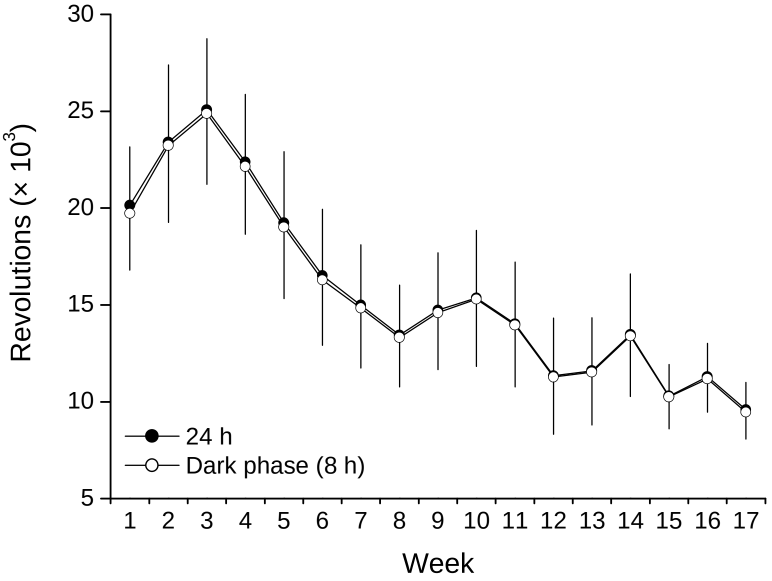

Figure 5.11. Amount (weekly mean) of wheel-running activity per day (+SEM) and per dark phase (−SEM) of eight hamsters during prolonged exposure to artificial long days.

In contrast to the light phase oxygen consumption of RW hamsters in the dark phase was significantly elevated (~50%) compared to controls without wheel (Figs. 5.12; 5.13), resulting in a higher daily O_2 uptake (Table 5.2). Correspondingly, relative food intake was increased in RW hamsters. Total daily food consumption, however, was not significantly elevated (*P* = 0.072; Mann-Whitney rank sum test) during the 4-day measurement when body weights of both groups were similar. The respiratory quotient was not different between the groups but significantly decreased in the exercising animals during the dark phase compared to the light phase. For the 4-day measurement the mean amount of wheel running was 21,413 ± 3,850 revolutions per dark phase (range 8,135-36,868) which corresponds to 99.5 ± 0.2% of the total amount of daily wheel revolutions. Thus, running exercise was almost completely restricted to the artificial night (Figs. 5.11; 5.13).

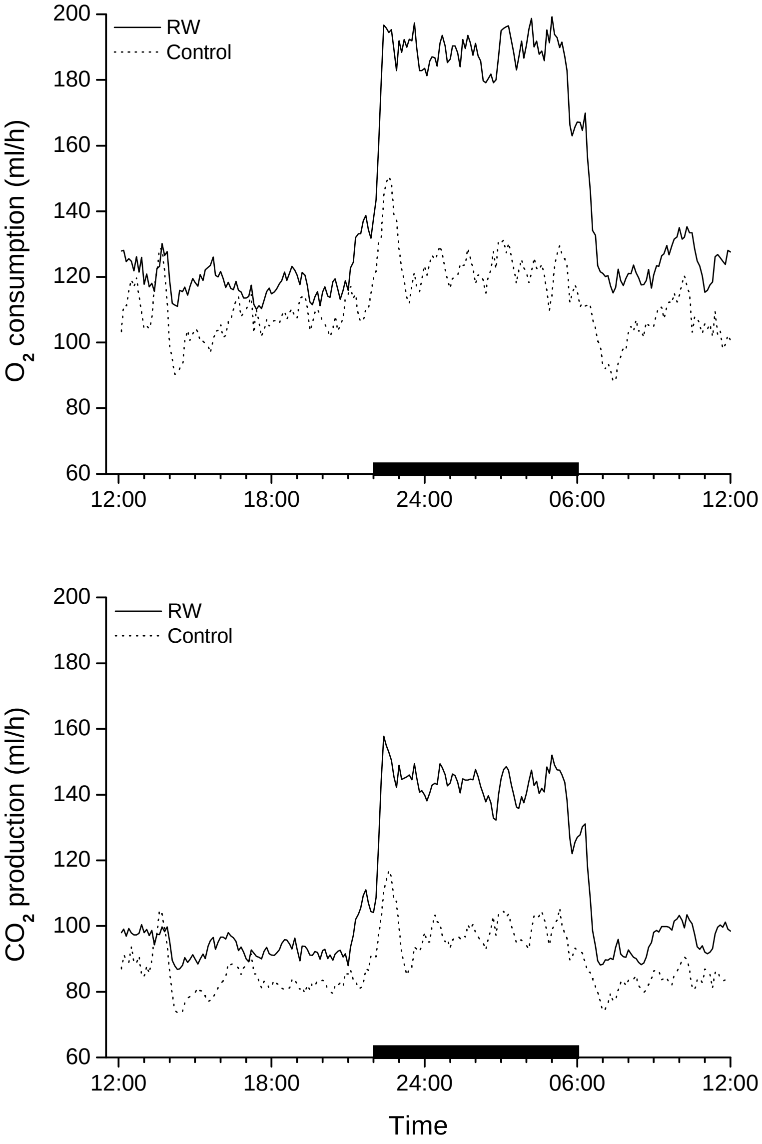

Figure 5.12. Oxygen consumption (A) and carbon dioxide production (B) during the course of the day (24 h) of hamsters with access to a running wheel ($n = 8$) and without ($n = 7$). Values are means of an averaged 4-day measurement for each animal. The dark phase (8 h) is indicated by the black horizontal bar.

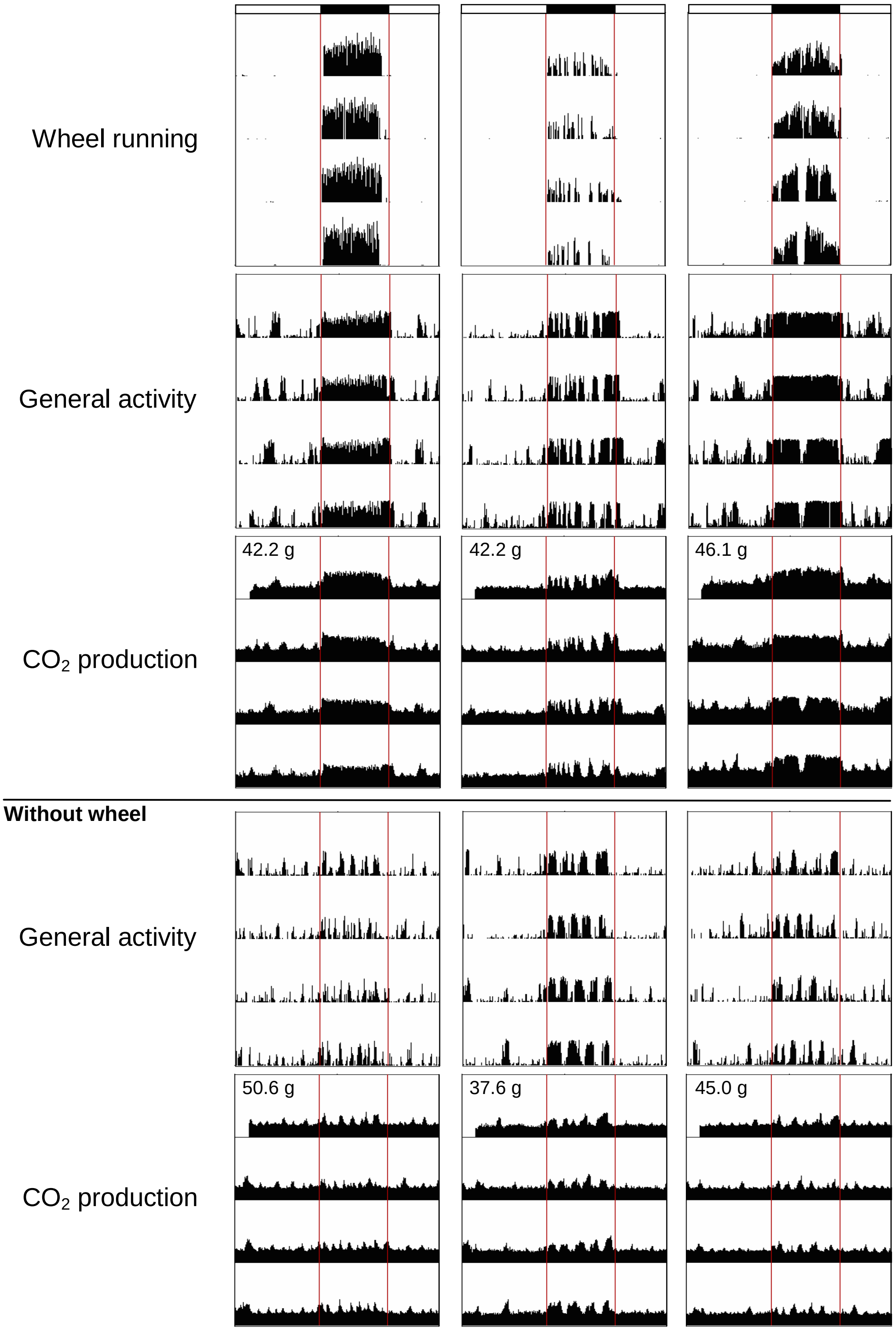

Figure 5.13. Activity and total CO_2 production of six individuals either with access to a running wheel (upper columns) or without (below the line). Body mass is given as mean of the initial and final value of the 4-day measurement. The dark phase (8 h) is denoted by the black area of the bar at the top and additionally by vertical lines, respectively.

Table 5.2. Mean oxygen consumption, carbon dioxide production, respiratory quotient and food intake of a 4-day measurement under long-day conditions (16 h of light, 8 h of darkness).

			RW (*n* = 8)	Control (*n* = 7)
Body mass (g)	initial		45.2 ± 5.2	44.8 ± 4.4
	final		45.2 ± 5.9	44.6 ± 4.2
O_2 consumption	absolute (ml/h)	light phase	122.5 ± 22.0	106.7 ± 7.4
		dark phase	187.0 ± 33.0^{d}	123.9 ±
		24 hours	144.0 ± 24.6	112.4 ± 7.4^{a}
	relative (ml/g · h)	light phase	2.73 ± 0.48	2.40 ± 0.16
		dark phase	4.19 ± 0.92^{d}	2.79 ± 0.17^{b,c}
		24 hours	3.22 ± 0.61	2.53 ± 0.25^{a}
CO_2 production	absolute (ml/h)	light phase	95.6 ± 13.8	84.0 ± 7.6
		dark phase	142.3 ± 19.0^{d}	98.3 ± 7.2^{b,c}
		24 hours	111.2 ± 14.6	88.8 ± 7.1^{b}
	relative (ml/g · h)	light phase	2.03 ± 0.14	1.89 ± 0.11
		dark phase	2.98 ± 0.16^{d}	2.21 ± 0.13^{b,c}
		24 hours	2.35 ± 0.47	2.00 ± 0.19^{a}
RQ (CO_2/O_2)		light phase	0.80 ± 0.05	0.81 ± 0.02
		dark phase	0.77 ± 0.04^{c}	0.81 ± 0.02
		24 hours	0.79 ± 0.05	0.81 ± 0.02
Food intake	absolute (g/d)		4.72 ± 0.76	4.02 ± 0.25
	relative (g/d · g		0.104 ± 0.009	0.091 ± 0.010^{a}

Values are means ± SD; for calculation of relative values the initial and final body mass were averaged; significant differences compared to the RW group are indicated by ^{a} ($P < 0.05$) and ^{b} ($P < 0.01$) (Mann-Whitney rank sum test); significant differences compared to the light phase within the group are indicated by ^{c} ($P < 0.05$) and ^{d} ($P < 0.01$) (Wilcoxon signed rank test).

DISCUSSION

Experiment 1

Under SD conditions exercising hamsters considerably increased their body mass. In contrast, the sedentary controls exhibited the normal decline in consequence of exposure to a short photoperiod. According to body composition data and plasma leptin results of the previous study (chapter 4), one has to assume that not only the decrease in body mass but also the exercise-induced increase was accompanied by a reduction in the relative fat mass (Wade and Bartness 1984, Klingenspor et al. 2000). Nevertheless, during the period of locked RWs the RW hamsters' body mass considerably decreased, demonstrating the potential for a body mass reduction despite the presumably low relative fat mass. This might be seen as support for the study of Klingenspor et al. (2000) that revealed a SD-mediated decrease in body mass almost equally due to a reduced fat and lean mass, contradicting the earlier findings of an unaffected lean body mass (Wade and Bartness 1984). On the other hand, the body mass reduction in RW hamsters (locked wheels) quite in parallel to that of controls (never with wheel) impressively shows a lasting effect of wheel running on body mass.

A further winter trait, daily torpor, usually occurs spontaneously only after about 10-12 weeks of SD exposure when the body weight has almost reached the nadir (Bartness et al. 1989, Ruf et al. 1993) which is linked to a marked depletion of fat stores. Within the RW group (wheels locked) torpor occurred the first time about one week later and overall in only three individuals compared to sedentary controls that showed torpor without exception. These results, together with the fact that torpor has never been observed in a hamster with access to a RW (see chapter 4), point to the impact of pronounced running exercise on this winter trait. In this, two different inhibiting effects seem to be present. On the one hand, the running behaviour obviously prevents torpor acutely. On the other hand, the present data suggest a lasting effect linked to the increased body mass, i.e. without a direct influence of wheel-running activity (wheels were locked). Accordingly, the three RW individuals that showed torpor in the present study reached a significantly lower body weight than the remaining RW animals at the end of the decline due to locked RWs during SD exposure. In this regard, it should be mentioned that two RW hamsters that were not observed being torpid showed a comparable absolute weight loss to those in which torpor occurred. One individual (the heaviest of all) reduced its body mass from 51.0 to 41.0 g. Despite this considerable loss of 10 g the animal's weight remained distinctly higher-than-average for a male winter hamster, and the relative weight loss (19.6%) was still below the values of the hamsters that displayed torpor (21.8-28.5%), even though only a little. The second individual reduced body mass from 44.6 to 35.8 g (−19.7%) and no torpor was observed in contrast to its sibling in which body mass decreased from 44.9 to 32.1 g (−28.5%). These results are in accordance with the hypothesis that the typical SD-mediated reduction in body mass is a prerequisite for the incidence of torpor in Djungarian hamsters (Dark et al. 1999, Freeman et al. 2004b). Within the search for the presumable weight loss-dependent physiological change that is permissive for torpor display, the adipose-derived hormone leptin (Zhang et al. 1994) was previously considered a potential candidate (Freeman et al. 2004b) because of its obvious role as an indicator for the body fat level in mammals. Strong correlations between body fat and leptin concentrations in blood were found in humans and several rodents (for Review, see Ahima and Flier 2000) including *Phodopus sungorus* (Klingenspor et al. 2000). The existing results concerning a potential torpor-inhibiting effect of leptin (Freeman et al. 2004b, Schmidt et al. 1997, chapter 4) are controversial and have been previously discussed with view to RW hamsters in chapter 4. Apart from leptin, changes in energy metabolism such as circulating concentrations of metabolic fuels in exercising hamsters are conceivable to be involved in inhibiting torpor, as already supposed by Thomas et al. (1993). In this context, the concentration of blood glucose, which comes to mind first, generally seems not to play a key role in inducing torpor. Namely, glucose concentrations are not lowered before but during hypothermia, and administered insulin does not provoke torpor bouts in LD hamsters (Dark et al. 1999). On the other hand, torpor is readily induced in LD hamsters treated with 2-deoxy-D-glucose that disrupts glycolysis (Dark et al. 1994, 1996). The authors concluded that the different effect is due to the general glucoprivic actions of 2-deoxy-D-glucose affecting specific neural targets. Hence, the search for the potential metabolic substrate inducing torpor is still on.

A causal relation between testis regression and the display of torpor was shown in castrated Djungarian hamsters where exogenous testosterone almost completely inhibited the incidence of controlled hypothermia (Bartness et. al. 1989, Vitale et al. 1985). In the present study, however, elevated blood testosterone can be ruled out as explanation for the low torpor frequency in RW hamsters. Although their testes were not palpated prior to the second period of free RW access, it has to be assumed that testis involution was at least almost completed. This is strongly suggested by the results of the previous study (chapter 4) where the testes of RW hamsters with uninterrupted RW access invariably reached a non-palpable state in winter. Following the results of Schlatt et al. (1999) such a non-palpable state is reached when each testis is < 50 mg. Thus, in the current study PTW of more than 200 mg after 15 weeks in SD are very unlikely but would have been a prerequisite for serum testosterone considerably elevated compared to lowest SD levels (Schlatt et al. 1995). Determination of final PTW of RW animals after the second period of exercise, as shown in Fig. 5.14, revealed only one PTW value below 100 mg.

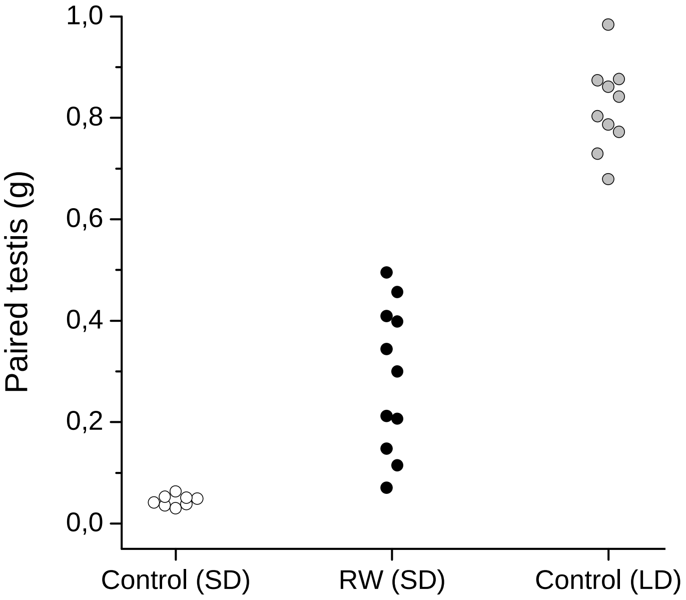

Figure 5.14. Paired testis weight after prolonged SD exposure (18 weeks) of hamsters with and without access to a running wheel, and LD controls without RW access. For mean values, see Table 5.1.

With respect to the previous study (see chapter 4) relatively high PTW reflect an advanced recrudescence due to wheel running rather than incomplete testicular atrophy compared to sedentary controls. In this regard, it should be added that one of the three RW individuals that definitely displayed torpor showed a notable PTW of 456 mg at the end of the experiment, i.e. four weeks after its last torpor bout. Together with the presumption that the testes of RW hamsters also underwent complete regression, the results of palpation during SD acclimatisation indicate a decelerated course of involution. Hence, together with the previous finding of an advanced recrudescence in exercising hamsters compared to sedentary controls (chapter 4), the duration of involuted testes appears to be shortened due to wheel running. If the follicle stimulating hormone (FSH) plays a crucial role in the testis cycle, as proposed by the results of Schlatt et al. (1999), its release from the pituitary gland might be altered by wheel running, i.e. a deceleration of the blood FSH decrease at the beginning of SD acclimatisation and an earlier increase in FSH secretion, resulting in advanced testicular recrudescence.

The unaltered change in fur colouration in RW hamsters provides further evidence for involuted testes, because exogenous testosterone (chronic implants) was shown to delay and attenuate the occurrence of this SD trait (Duncan and Goldman 1984a). Furthermore, the present results suggest that the seasonal change in circulating prolactin levels, underlying the seasonal fur colour changes (Duncan and Goldman 1984b), remained unaffected by running exercise since there was no difference in the colour change between (temporarily) exercising and sedentary hamsters.

It is possible that torpor events occurred without having been registered, since the observation was not carried out every day. However, the probability that a RW individual displayed, e.g., two bouts that were both overlooked is less than 10%. In any case, 30 days of observation out of 43 days (~70%) were considered sufficient to provide a reliable sample size for comparison of torpor frequency as shown in Fig. 5.3.

Not only daily torpor, changes in fur colouration, and gonadal regression, but also the reduction in body mass contributes to saving energy in winter when thermogenic costs massively increase. This striking weight loss (up to ~40%) is associated with distinct changes in body composition. A significantly elevated relative heart weight in SD-acclimatised Djungarian hamsters compared to summer hamsters was previously shown in a natural photoperiod with either natural or constant T_a (23°C), demonstrating the photoperiod as a major trigger (Puchalski and Heldmaier 1986). This finding is not surprising since SD is the decisive signal for the winter traits in *Phodopus sungorus*, including the reduction in body mass which obviously is not paralleled by a decreasing heart weight, and thus accounts for the increase in relative heart weight. The phenomenon of a raised relative heart weight in winter has been demonstrated for different small rodents such as *Apodemus sylvaticus*, *Pitymus duodecimcostatus* (Pérez-Suárez et al. 1990), *Apodemus flavicollis* and *Clethrionomys glareolus* (Klaus 1988). The latter study revealed that in both species elevation in relative heart weight is not due to the change in photoperiod, but a response to cold. Irrespective of whether evoked by changes in photoperiod or $T_{a,}$ the seasonal change in relative heart weight probably reflects higher O_2 demands during winter. The increased cardiac output per gramme tissue in winter can be accompanied by changes in haematological values such as the number of red blood cells (RBC), their mean corpuscular volume (MCV) or mean corpuscular haemoglobin (MCH). In Djungarian hamsters the haemoglobin concentration of the blood is nearly unaffected by (the) season, but RBC is increased in winter, providing an enlarged area for gas exchange (Puchalsky and Heldmaier 1986). The lack of seasonally varying haematocrit (Hc) in the latter study is contradicted by results of the third experiment in chapter 4 (see suppl. Fig. S.2). There the hamsters exhibited clearly increased Hc in winter compared to the following summer, consistent with the higher oxygen demand in the cold. Despite pronounced exercise RW hamsters displayed a lowered relative heart weight compared to sedentary controls after 18 weeks in SD. This is in accordance with the assumption that the elevated relative heart weight of SD-acclimatised hamsters is mainly due to the loss in body mass without reducing the heart weight. Since RW animals increased body mass instead of the typical reduction, the relative heart weight was comparable to those of equiponderate LD controls. However, compared to the latter group there was a clear tendency of higher relative heart weights in RW hamsters (n.s.; Fig. 5.6), indicating a slight effect of exercise rather than of photoperiod. In the previous study the mean relative heart weight of the exercising and sedentary group in summer was similar (suppl. Fig. S.5) which contradicts an effect of wheel running. However, it is worth mentioning that the free access to RWs already lasted almost nine months, and it was shown that the amount of wheel running considerably decreases with time (Fig. 5.11; Fig. 3.6). Thus, endurance performance might have already been too low to affect the heart weight. Unlike the relative heart weight the relative liver weight in SD was similar for exercising animals and sedentary controls in the present study, but elevated compared to LD controls. Since the liver plays a crucial role in energy metabolism the finding probably reflects an evolved adaptation to severe cold that demands an increased metabolic rate in winter hamsters (represented by SD controls) on the one hand, and on the other hand a response (e.g., glycogen content) to the pronounced exercise in RW hamsters. In this regard, the relative kidney weight, which was similar for all groups, might serve as a kind of reference organ, on average strongly correlated with body mass. The elongated mean femur length of RW hamsters compared to SD controls provides further evidence for running-induced growth, already suggested by the results of the previous study (chapter 4). LD controls exhibited a similar mean body mass as RW animals but had on average longer femora, demonstrating that the stimulation of growth due to temporary exercise in SD did not compensate for the achievement of the LD state. This directs attention to the body composition which was already shown to be different between both groups at least with respect to the liver. Moreover, since wheel running decreases the relative fat mass (see chapter 4), an increased lean mass in exercising animals is not only indicated when related to body mass but also with respect to the skeleton size compared to the fat summer hamsters with longer femora.

Experiment 2

Inhibition of the typical SD-mediated body weight reduction (~30%) due to wheel running is in accordance with the results of the previous investigation (see chapter 4). However, a significant loss in body mass was observable in the RW hamsters of the present study, indicating that the weight-reducing influence of the shortening photoperiod was still present and in conflict with the growth-stimulating effects of running exercise. Moreover, the latter effect did not result in a significantly elevated body mass in RW animals compared to controls after one year, when both groups reached the high summer level, which was also different to the previous results. This might be explained either by an increased reduction in fat mass compared to the RW group of experiment 3 in chapter 4 (Fig. 4.1C) or by a less pronounced growth stimulation. Since there were no sedentary controls exhibiting the normal seasonal cycle of body mass twice/a second time, interpretation of the weight reduction after the exchange of the wheels is difficult. The considerably decreased response in reducing the body mass compared to the animals that had no RW access during the first year might either reflect the (lasting) consequences of growth-stimulating exercise or simply a damped SD acclimatisation in the second year. At least for the change in fur colouration such a lowered response to SD is evident during the animals' second winter. Anyway, the potential of losing body mass due to SD after removal of the wheels supports the findings of experiment 1 (locked wheels), indicating the fact to be negligible whether RWs are locked or removed.

According to the findings of Hoffmann (1978) the increase in body mass in sedentary juvenile hamsters was discontinued in SD, and in the present case it happened when the body weight of the older, SD-acclimatised controls has been reached. This fact, together with the subsequent congruency of body weight courses of both control groups impressively demonstrates the ‘sliding set-point’ (introduced by Mrosovsky and Fisher 1970) that was previously described for the SD-mediated decline in this species (Mercer et al. 2001, Steinlechner et al. 1983). It is very likely that growth is interrupted when hamsters respond to an ‘inhibitory’ photoperiod with either a considerable reduction in body mass or a discontinued increase (Fig. 5.8), which is associated with a delay in reproductive aging (Hoffmann 1978, Place et al. 2004). In common shrews (*Sorex araneus*) seasonal changes were not only found for body weight and dimension (head and body length; changes in intervertebral discs) but also for organs (even the brain; Pucek 1965) and the skull (braincase depth; ‘Dehnel’s phenomenon’) with lowest values during winter (Dehnel 1949, Pucek 1970), proving even a shrinkage of bone dimensions. However, the described seasonal change of body mass in shrews appeared to be mainly (> 80%) the result of changes in tissue hydration (Myrcha 1969).

Experiment 3

Body mass of RW hamsters significantly increased due to running exercise compared to sedentary controls that maintained an almost constant level, indicating a growth-inducing effect of wheel running under LD conditions. Despite the fact that significance for different changes in body mass was only reached after 13 weeks of RW access, the results are rather convincing because of the hamsters’ age. At the beginning of the experiment the animals were already about one year old, and prior to RW access (for one group) the body weights were over 40 g, i.e. roughly the typical maximum of aged male Djungarian hamsters, at least in our breeding colony. Consistently, control animals did not further increase their body mass in the course of the experiment. Hence, body weight elevation in RW hamsters appeared, although growth, which slows down considerably after the juvenile phase (in LD), presumably was completed. In any case, the experiment revealed a wheel running-induced effect on body mass that closely resembles those shown in Syrian hamsters (*Mesocricetus auratus*; Gattermann et al. 2004).

Within the first three days of RW access the mean body mass of exercising hamsters decreased by 2 g ($P < 0.05$; Wilcoxon signed rank test), probably reflecting a negative energy balance as a result of increased locomotor activity without an appropriate adjustment of food intake. This tendency of either an initially reduced body weight due to daily exercise or a short-term deceleration of the normal juvenile body mass increase (Fig. 5.7) has already been observed in previous experiments, and was also shown in

Syrian hamsters (Rowland 1983). Rowland assigned his results to previous reports about similar findings which were interpreted as evidence for the hamsters' food intake to be relatively inflexible in responding to weight loss. To return to the present results, the initially reduced body mass of RW hamsters remained at the lowered level for about two weeks prior to the gradual rise. In this connection the question arises whether this was due to a decrease of fat mass, which was previously shown to be one consequence of wheel running (chapter 4). In any case, the decreased RQ of RW hamsters during the dark phase, when the animals are exercising, indicates a higher proportion of metabolised lipids than in the light phase, i.e. the rest phase. This is well in accordance with the expected adjustment of energy metabolism during prolonged exercise. Hence, the lack of intraday RQ changes in control animals seems to reflect a moderate activity level in the dark phase that does not require a change in the ratio of metabolised substrates (Fig. 5.13). Surprisingly, the absolute daily food intake was not significantly elevated in RW animals during the 4-day measures of the metabolic rate. However, related to body weight food consumption was higher in exercising hamsters. Considerable variations in body weight underlying the mean value, which was quite similar for both groups, contribute to the variability of absolute amounts of daily food intake, and thus, prevent potential differences being revealed. Allowing for this problem weight-specific values were calculated, bearing in mind that those can only serve as approximations. In the course of the entire experiment the relative food intake considerably decreased in RW hamsters. Despite the concomitant increase in body mass it is likely that the decreased relative food consumption was rather related to reduced running exercise. For instance, Syrian hamsters were shown to double their total daily food intake due to wheel running within four weeks (Gattermann 2004) which, however, contrasts to the tendency of earlier results (Rowland 1983). This inconsistency, however, might be explained by short duration of the experiments lasting only for about four weeks. With view to energy expenditure in the present study, relative daily O_2 uptake of RW hamsters was about 27% higher compared to sedentary controls, whereas daily food intake per gramme body mass was only elevated by about 14%. This discrepancy might indicate an improved utilisation of ingested nutrients in exercising animals. However, prior to O_2 measures the individuals had only one to two weeks of free access to a RW, i.e. a rather short period for an adjustment of digestion.

Final conclusions

To summarise, the present results strongly support the findings described and discussed in the previous chapter. There can be no more doubt concerning a growth-promoting effect of wheel running in SD conditions when compared to sedentary animals. This conclusion is based on 1) the relative kidney weight being similar for sedentary controls and larger RW hamsters 2), the RW hamsters' decline of body mass in parallel to the weight course in sedentary controls during the period of locked wheels, 3) the increased rate of regaining weight after releasing the wheels compared to the first period of free RW access, indicating a 'predetermined way' to an elevated set-point, and 4) the elongated femora of RW hamsters. The latter fact confirms not only the slight tendency of lengthened femora (~4%; n.s.) in the previous study with significance only for vertebral columns, but becomes more important with respect to the findings in Syrian hamsters. There, running-induced elongation of the vertebral column was more pronounced than that of femora (Borer et al. 1977). Furthermore, the increase in body mass in exercising hamsters housed under LD conditions probably reflects the same influence of wheel-running activity. Such an endurance performance undoubtedly increases energy expenditure in hamsters that additionally gain weight. However, accumulation of those additional energy demands was not associated with an adequate rise in food consumption. Indeed, one experiment provided a significantly increased relative food intake of RW hamsters but the other did not. This fact remains intriguing and, in this connection should attract notice in further investigations.

6

Discussion

The present study on wheel-running activity in Djungarian hamsters (*Phodopus sungorus*) revealed some striking findings that substantiate the notion of wheel running to be a distinct and special form of behaviour. For instance, in contrast to the general activity, running exercise was almost completely restricted to the dark phase which has already been shown for the related Syrian hamster (*Mesocricetus auratus*; Zucker and Stephan 1973). Thus, a masking effect of light on wheel running as previously demonstrated for Syrian hamsters (Redlin and Mrosovsky 1999) is also very likely in Djungarian hamsters. Most strikingly, wheel running affected different traits of the seasonal acclimatisation. Shallow daily torpor was completely inhibited in RW hamsters and the duration of involuted testes in short days was abbreviated, whereas the fur colour change remained unaltered. Moreover, the typical annual cycle of body weight was prevented by a growth-promoting effect of running exercise. Comparable effects on deep hibernation, the testis cycle and growth were previously found in Syrian hamsters again pointing to the relatedness between both hamster species despite their different strategy of coping with harsh winter conditions.

The different sections of the thesis are summarised below followed by perspectives resulting from the present findings.

Ambient temperature

Wheel-running activity was suppressed at thermoneutral ambient temperature (~26-27°C) compared to cooler T_as but was not further enhanced at ~15°C compared to ~22.5°C. This lack of negative correlation between the number of revolutions and the T_a contradicts the hypothesis that wheel running is used to maintain a high body temperature at a low ambient temperature, i.e. as a support for thermoregulation (Janik and Mrosovsky 1992). If this would be the case an elevated activity level could have been expected at the lowest T_a in the present study. In general, saving energy becomes more important with decreasing T_a. Therefore, heat production by the help of brown fat would be advantageous compared to wheel running where the spent energy is partially converted into locomotion. In the present study food was provided ad libitum, thus it presented no limitation to energy uptake. However, it remains speculative whether abundant food interferes with behaviours that contribute to saving energy at cold temperatures. Actually, Djungarian hamsters respond to prolonged exposure to SD and/or cold with increased thermogenic capacity of the brown adipose tissue (Heldmaier et al. 1981, Rafael et al. 1985a, b) and voluntarily reduce the food intake under SD (Knopper and Boily 2000, Steinlechner et al. 1983), reflecting both a strong influence of cold T_a as well as an inherent property unaffected by food abundance.

Indications of comparable effects of high T_a were also found by personal observations that suggest a suppression of wheel running at high T_as during summer in the study on the annual activity pattern (chapter 3). However, the attempt to show a correlation between T_a and wheel-running activity was inconclusive. If any influence of T_a existed, which is likely with respect to the first experiment (chapter 2, see also suppl. Fig. S.1), the strong individual daily variations in the amount of wheel running together with the annual course prevented it from being revealed, suggesting a focus on this issue in further experiments.

Annual activity pattern

The more pronounced activity rhythm of RW hamsters during autumn and winter is well in accordance with previous results that indicated a stabilising effect of wheel running on rhythm generation of the endogenous clock (Steinlechner et al. 2002b). This implicates that locomotor activity feeds back to the circadian pacemaker which is coherent with the prevailing opinion (Koteja et al. 2003).

The annual course of the amount of activity in hamsters without wheel indicates that general activity is highest around the autumnal equinox coinciding with potential food hoarding activity for the upcoming meagre times. Subsequently, the nocturnal rodents showed a gradual decline in locomotion and lowest activity in winter despite the lengthened dark phase. The following activity increase during the spring might reflect, among other factors, the end of reproductive quiescence. A spontaneous recurrence to high amounts in wheel-running activity after about 150 days of artificial SD exposure, i.e. at the time when the reproductive axis becomes refractory, was also found in castrated male Syrian hamsters (Ellis and Turek 1983). The fact that the hamsters were castrated indicates that the assumed relationship between photoperiod-induced state and behavioural change was independent of testosterone. In the present study wheel running failed to increase again subsequent to the low amounts in winter which suggests that the influence of diminished motivation to run in a wheel might have concealed the trend that was found in hamsters without RW access. This assumption in turn supports the notion of Sherwin (1998b) that wheel running and general activity are not directly comparable. Although the author finally even concluded that wheel running is a laboratory artefact he did not rule out an underlying natural behaviour that is enhanced or modified by running exercise. This latter possibility is strongly supported by the present results mainly based on similarities in the annual course of the activity amount of hamsters with and without a wheel.

Effects of running exercise

The present results provide evidence of a growth-stimulating effect that is independent of the seasonal state of the hamsters. Although the increase in body mass of RW hamsters kept under LD 16:8 was not proven to be a result of growth it is very likely and would confirm previous findings in exercising Syrian hamsters kept under LD 14:10 (Gattermann et al. 2004).

First it was surprising not to find a significant elevation in relative heart weights of RW hamsters despite the considerable exercise of several hours each day. Indeed, there was only a tendency of increased relative heart weights and only in one of two experiments. The explanation might be that the level of the endurance performance was overestimated. Both the results of food intake and oxygen consumption indicate only a moderate level of exercise. However, there is no doubt concerning the activity level being higher in hamsters with access to a running wheel than in sedentary control animals. This was convincingly reflected by a low fat mass, an increased relative liver weight and most strikingly by the growth-stimulating effect.

Perspectives

For investigation of the mechanism by which the stimulation of growth is triggered, SOCS3 might be an auspicious candidate. It was shown to be involved in leptin signalling within the arcuate nucleus of the hypothalamus. Since leptin not only plays a role in the regulation of the seasonal body weight cycle but was already associated with growth, it would be interesting to compare leptin sensitivity between RW hamsters and sedentary controls during short-day acclimatisation. If the short day-induced weight reduction in RW hamsters is prevented despite a low level of SOCS3 (sensitive to leptin) which should lead to a reduction in fat and body mass, then the effects of SOCS3 are not involved but are probably in conflict with growth. If, however, the SOCS3 level is altered in RW hamsters compared to the controls, the leptin sensitivity which is triggered by the SOCS3 level has to be assumed to play a crucial role in the observed phenomenon. Moreover, the regulation of daily torpor is still unknown. In this regard, the acute inhibiting effect of wheel running on the incidence of torpor might be helpful insofar as a model is provided in which hypotheses can be tested either with or without RW access. However, concerning torpor the first question that has to be answered is whether the acutely inhibiting effect of wheel running is evoked by an altered behaviour such as the sleep/wake cycle or is due to physiological changes.

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Supplemental data

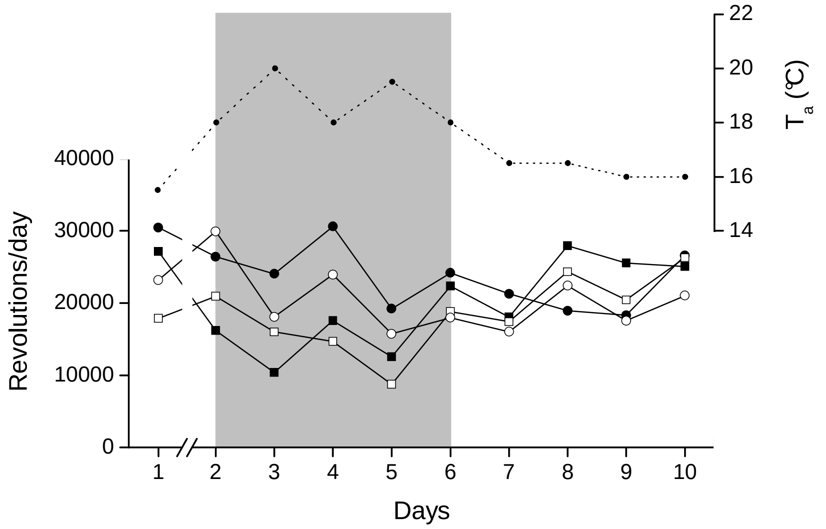

Figure S.1. Correlation between the daily amount of wheel running and ambient temperature (T_a; at 0400 h, CEST) in four hamsters kept outdoors under natural lighting conditions. Between the first day and the remaining nine consecutive days (end of June) one day is lacking due to missing data.

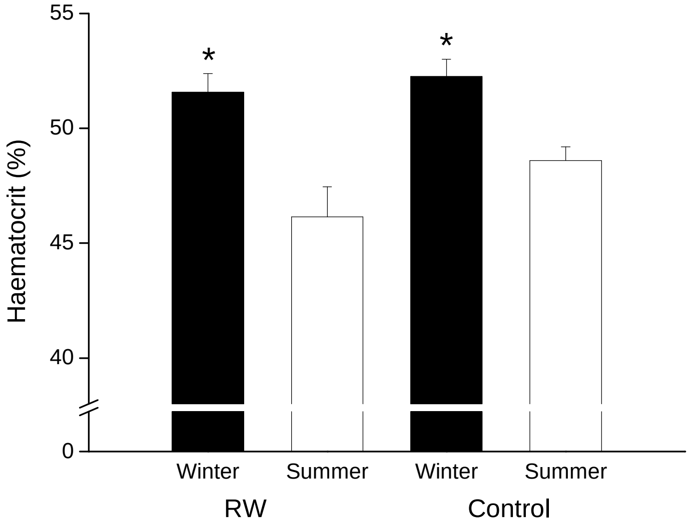

Figure S.2. Haematocrit values of hamsters with access to a running wheel ($n = 7$) and without ($n = 8$) in winter compared to the following summer. There is no significant difference between RW hamsters and controls. Values of RW animals were compared with Mann-Whitney rank sum test instead of Wilcoxon signed rank test (for repeated measures), because only six individuals were represented in both groups. Values are means + SEM; * Significant difference compared to summer ($P < 0.01$).

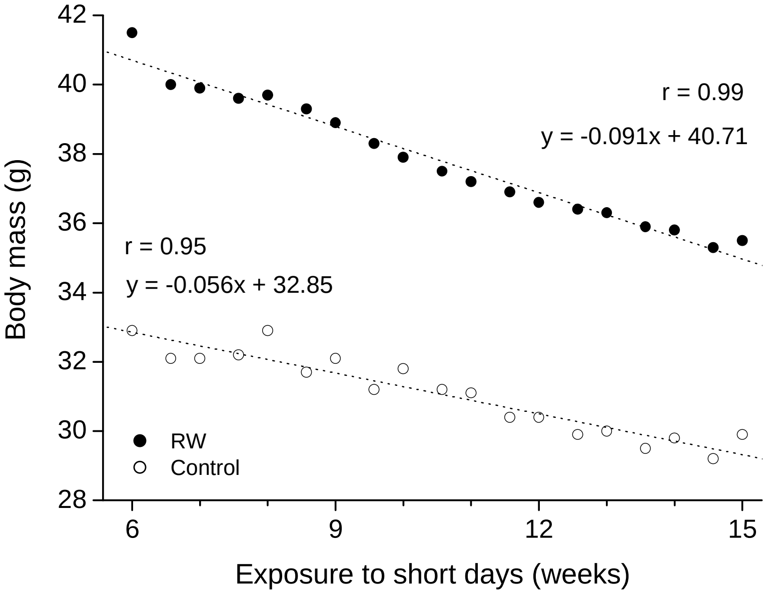

Figure S.3. Decrease in body mass of hamsters with blocked running wheels (*n* = 11) compared to control animals (*n* = 8) housed without wheels under artificial short-day conditions. Comparison of regression lines (Student's *t*-test) revealed no significant difference for regression coefficients.

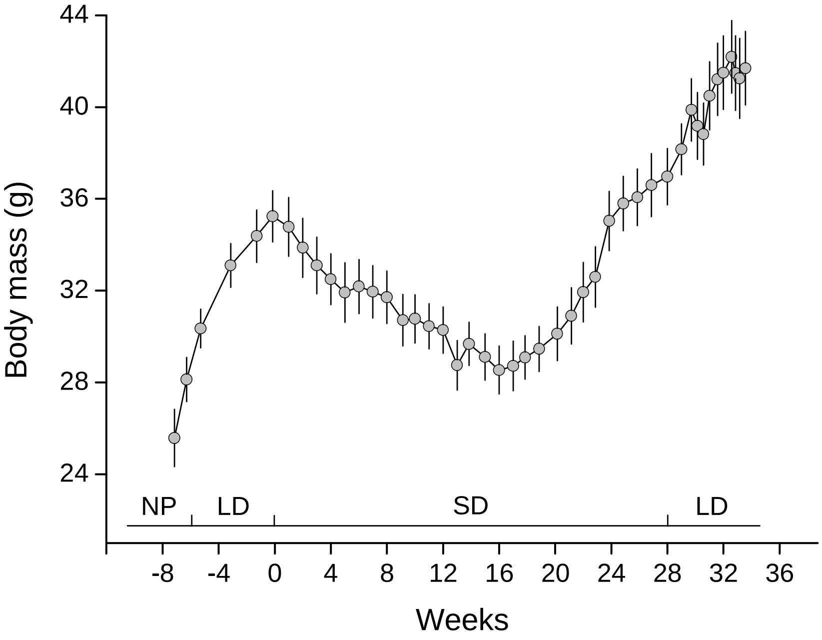

Figure S.4. Body weight course of hamsters (*n* = 10) that were used as long-day (LD) controls for measures of food intake and organ weights. For details of lighting conditions see chapter 5. Values are means ± SEM.

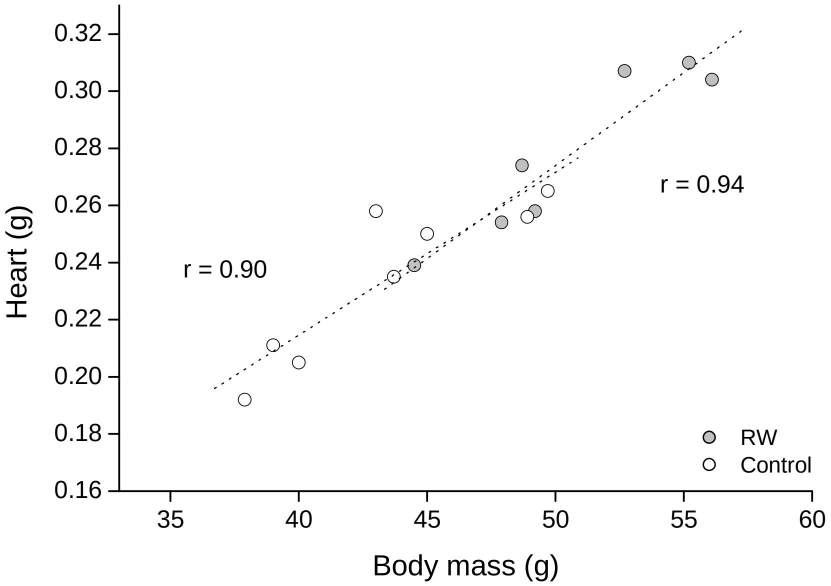

Figure S.5. Relation of heart and body mass in exercising (RW) and sedentary hamsters (14-16 months old). The animals were kept outdoors under natural daylight with natural T_a until summer (June); for details, see experiment 3. Linear regressions are highly significant (ANOVA; $P < 0.01$).

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