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Voluntary access to a warm plate reduces hyperactivity in activity-based anorexia

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Abstract

Activity-based anorexia (ABA) is considered an animal model of anorexia nervosa. In ABA, scheduled feeding in combination with voluntary wheel running leads to hyperactivity, reduced food intake, severe body weight loss and hypothermia. In this study it was investigated whether hyperactivity in ABA could be reduced by introducing a warm plate (which was voluntary accessible and did not influence ambient temperature) into a part of the cage.

In ad libitum fed rats, the presence of the warm plate did not influence body temperature, running wheel activity (RWA), body weight or food intake. During ABA, however, rats preferred the warm plate and hypothermia was prevented, while hyperactivity and body weight loss were significantly reduced when compared to ABA rats without a plate. Correlation analysis revealed a significant association between basal body temperature and RWA during the light phase in ABA rats. However, there was no evidence that initiation of light phase RWA was a result of hypothermia. These data suggest that ABA rats prefer to prevent hypothermia passively by choosing a warm plate rather than actively regulating body temperature by hyperactivity.

Keywords: Temperature; Anorexia; Hyperactivity; Animal model; Running wheel activity

1. Introduction

Anorexia nervosa is a psychiatric disorder often characterized by extreme hypophagia, body weight loss and hypothermia. Patients frequently show excessive exercising and can not avoid being active [1,2]. The activity-based anorexia (ABA) model is used to study anorectic behavior in rodents and serves as an animal model of anorexia nervosa. In ABA, voluntary access to a running wheel in combination with scheduled feeding leads to a paradoxical increase in running wheel activity (RWA) and a decrease in food intake, resulting in substantial body weight loss (>20%). Not only total RWA increases, but the distribution of activity throughout the day changes as well. ABA rats also show hypothermia, loss of estrous cycle, stomach ulceration and will eventually die of emaciation [3,4].

The biological trigger of hyperactivity in ABA rats remains unclear. Hyperactivity of ABA rats might be explained by anticipation to the feeding period [5]. Indeed, substantial activity takes place prior to food access (independent of light or dark phase), which is also known as food-anticipatory activity (FAA). Wheel running is also considered as foraging behavior and has rewarding properties [6,7] and recently it was hypothesized that reduced plasma leptin levels associated with body weight loss might trigger hyperactivity [8]. Furthermore, hyperactivity might be explained as a thermoregulatory behavior to prevent starvation-induced hypothermia [9], since activity levels of ABA rats are inversely related to core body temperature [10].

Thus far, only a few reports focused on whether body temperature affects ABA, by influencing ambient temper-

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ature. From these reports it appeared that ambient temperature has a strong influence on survival rate in ABA. Low ambient temperatures increase RWA and enhance the development of ABA, whereas high ambient temperatures, reduce (but do not prevent) RWA and thereby inhibit the development of ABA [9]. Although wheel running of ABA rats can be altered by manipulating ambient temperature, the effects obtained are probably not specific for ABA, since ad libitum fed running rats also decrease RWA when housed in a warmer environment and increase RWA in a colder environment [11–14].

The hypothermia observed in ABA rats might be perceived as unpleasant and this may trigger hyperactivity. Therefore, an experimental setting was designed in which rats could choose for a warm plate in order to passively raise body temperature, instead of active behavioral temperature regulation via RWA. In the present experiment rats had voluntary, instead of forced, access to a warm environment, which was provided by a warm plate in the cage that did not influence ambient temperature. It was hypothesized that ABA rats, but not ad libitum fed running rats, would prefer a warm environment and that the presence of a warm plate in the cage would subsequently reduce RWA in ABA rats.

2. Material and methods

2.1. Rats

Female outbred Wistar WU rats (Harlan, Horst, The Netherlands) weighing 180 g upon arrival were individually housed in a temperature and humidity controlled room (21±2 °C) under a 12:12 h light/dark cycle (ZT12=lights off). Rats were allowed to adapt to these housing conditions for 1 week under ad libitum food and water conditions. The ethical committee on use and care of animals of Utrecht University approved all described procedures.

2.2. Surgical procedures

One week after arrival all rats ($n=13$) received transmitters (TA10TA-F40 Data Sciences International, St. Paul, MN, USA) in the abdominal cavity under fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, 0.1 ml/100 g im) and midazolam (Dormicum®, Hoffman-LaRoche, Mijdrecht, The Netherlands, 0.05 ml/100 g, i.p.) anaesthesia. After surgery, rats were treated with buprenorphin (Temgesic®, Schering-Plough, Maarssen, The Netherlands, 0.05 ml/100 g, s.c.) and saline (1 ml, s.c.) and were allowed to recover for 2 weeks.

2.3. Experimental set-up

After 2 weeks of recovery from surgery, rats were housed in cages with running wheels for a free training period of 15 days (day-15–day 0) with ad libitum food and water access. RWA was continuously registered using a Cage Registration Program (Dep. Biomedical Engineering, UMC Utrecht, The Netherlands). On day-7 rats were divided into two groups (warm plate $n=7$/no plate $n=6$) matched for RWA (average 6077.7±977.0 revolutions at day-7) and aluminum plates (covering ±20% of cage area) were introduced into seven cages. Plates were separated from the sawdust by a Perspex layer and were heated to 37 °C by a continuous flow of hot water using a covered water bath. It was confirmed that ambient temperature in the cages was not affected using a (non-contact) thermometer. On day-2, transmitters were activated for baseline measurements of body temperature. Food was removed from all cages at the onset of the dark period of day 0 (ZT12). The next days (days 1–6) rats had 1 h access to food at ZT12, while water was continuously available. Body weight (just before ZT12) and food intake (ZT13) were measured daily. On day 6 (ZT11) rats were decapitated. Trunk blood was collected in lithium-heparin (Sarstedt, Nümbrecht, Germany) containing tubes after adding 83 μmol EDTA and 1 mg aprotonin. Plasma was frozen at −20 °C. Interscapular brown adipose tissue (BAT) and white adipose tissue (WAT) surrounding the ovaries and oviduct were weighed and stored at −80 °C.

2.4. Radioimmunoassay

Plasma levels of leptin were measured using a commercially available rat leptin RIA kit (sensitivity: 0.5 ng/ml), according to the manufacturer's protocol (Linco Research, St. Charles, Missouri, USA).

2.5. Quantitative PCR

Total RNA was prepared from BAT and WAT of ABA rats using Trizol Reagent (Invitrogen Gibco, Paisley UK). RNA was treated with DNAse I and was reverse transcribed into cDNA using oligodT_{12-18} primers and SuperScript II reverse transcriptase (Gibco). The Lightcycler real time PCR detection system (Roche Diagnostics, Mannheim, Germany) was used for amplification and quantification of uncoupling protein (UCP1) and leptin mRNA expression levels. Cyclophilin was used as a reference gene. An amount of cDNA corresponding to 40 ng of total RNA was amplified using the Lightcycler-Faststart DNA Master SYBR Green I kit (Roche Diagnostics) and the appropriate primers. Optimal $MgCl_2$ concentrations, annealing temperature and cDNA dilution (1:10) were determined (Table 1), resulting in PCR efficiencies >1.8. All cDNA samples were measured in duplicate. Expression levels of UCP1 and leptin were calculated as a normalized ratio relative to a calibrator sample (Rn). Thus, UCP1 and leptin expression were analyzed relative to cyclophilin and each sample was

Table 1
Quantitative PCR characteristics

Gene		Primer sequences	$MgCl_2$ (mM)	Annealing temp. (°C)	Product size (bp)	GenBank accession no.
Leptin	Forward	CCTGTGGCTTTGGTCCTATCT	3	61	241	NM_136076
	Reverse	CAAGCTGGTGAGGATCTGTTG				
Uncoupling protein 1 (UCP1)	Forward	CCACATAGGCGACTTGGA	4	63	79	NM_012682
	Reverse	TTCGTGGTCTCCCAGCATAG				
Cyclophilin	Forward	ATGTGGTCTTTGGGAAGGTG	4	59	161	NM_022536
	Reverse	GAAGGAATGGTTTGATGGGT				

normalized to a calibrator sample (diluted pooled cDNA of ad libitum fed rats), which was run in the same experiment (Roche Diagnostics).

2.6. Warm plate preference

To validate warm plate preference (versus preference of other cage locations), a subset of rats ($n=3$) with a warm plate in their cage was analyzed by video monitoring using the Observer system (Noldus Information Technology, Wageningen, the Netherlands). The cage was divided into three zones (wheel, warm plate and residual zone) and the presence of the rats in the three zones was analyzed during 2 h in the light phase (ZT9 to ZT11) during ad libitum feeding (days-2 and -1, thus after 5 days of habituation to the warm plate) and during ABA (days 3 and 4).

2.7. Data analysis

All data are presented as mean ± standard error. Data were analyzed using SPSS 11.5 for Windows and were controlled for normality and homogeneity.

Body temperature and locomotor activity (LMA, in rats without a plate) were measured using telemetry (bin size 10 min). Basal body temperature was analyzed as average body temperature during at least 30 min of inactivity in the early light phase (ZT0–ZT3). RWA, relative body weight, food intake, and basal body temperature were analyzed by GLM repeated measures analysis (time = days, treatment = warm plate/no plate) using Huynh Feldt correction for Mauchlys sphericity effects. Baseline measurements of body temperature, food intake and body weight were performed on day-2 and day-1. Baseline measurements of RWA were performed on day-4 to day-1. Final body weight, cumulative (days 1–6) food intake, fat pad mass, plasma leptin levels and qPCR data of ABA rats were analyzed by *T*-tests. Warm plate preference during ad libitum feeding and ABA was averaged over 2 days and analyzed by ANOVA and Wilcoxon signed-rank test for two-related samples. Pearson correlation analysis (days 3 and 4) was performed to investigate associations between body temperature, RWA and relative body weight. Differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Warm plate preference

Ad libitum fed running rats showed no preference for the area of the cage where the warm plate was located (following 5 days of habituation to the plate) ($F(2,8)=4.52$, n.s.).

However, during ABA, the same rats developed a clear preference for the area containing the warm plate as compared to other areas in the cage (including the running wheel) ($F(2,8)=103.49$, $p<0.001$). As a result, rats spent significantly more time on the warm plate during ABA than during ad libitum feeding conditions ($z=-1.60$, $p=0.05$). During ABA, rats with a warm plate also spent significantly more time in the running wheel ($z=-1.60$, $p=0.05$) and spent significantly less time in the remaining parts of the cage ($z=-1.60$, $p=0.05$) when compared to ad libitum feeding (Fig. 1).

3.2. Effects on body temperature

In ad libitum fed running rats, body temperature was not influenced by the presence of a warm plate in the cage ($t(10)=-1.59$, n.s.).

In ABA rats, body temperature decreased during the light phase. This starvation-induced hypothermia was significantly inhibited in rats with a warm plate as compared to

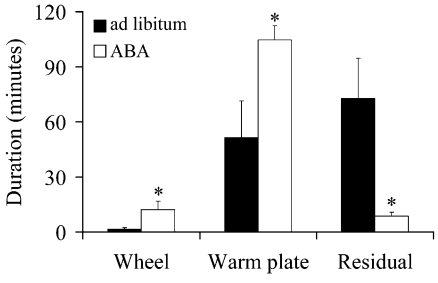

Fig. 1. Warm plate preference in ad libitum fed running rats and activity-based anorexia (ABA) rats. Warm plate preference was analyzed in rats during ad libitum feeding (black, average days-2 and -1) and during ABA (white, average days 3 and 4). The warm plate covered ±20% of the total cage area. The presence of rats in three zones (running wheel, warm plate or residual) in the cage was analyzed by video monitoring during 2 h in the light phase (ZT9 to ZT11). Data were analyzed by Wilcoxon signed-rank test for two-related samples (1-tailed), $*p \leq 0.05$.

rats without a plate (day: $F(6,66)=24.93$, $p<0.001$, day × treatment: $F(6,66)=8.30$, $p<0.001$) (Fig. 2).

Interscapular brown adipose tissue (BAT) mass remained significantly higher in rats with a warm plate as compared to rats without a plate after 1 week of exposure to ABA ($t(11)=-2.86$, $p=0.02$) (Table 2). UCP1 expression in BAT was 3.3 fold increased in ABA rats without a plate as compared to ABA rats with a warm plate, however this effect was not significant due to large variations in UCP1 expression in both groups ($t(10)=1.60$, n.s.).

3.3. Effects on running wheel activity

RWA of ad libitum fed rats (day: $F(3,33)=1.99$, n.s., day × treatment: $F(3,33)=0.27$, n.s.) was not significantly affected by the presence of a warm plate.

During scheduled feeding, total RWA was significantly different between rats with a warm plate and rats without a plate. RWA during the light phase (day: $F(6,66)=12.99$, $p<0.001$, day × treatment: $F(6,66)=4.22$, $p=0.01$) as well as during the dark phase (day: $F(6,66)=2.39$, $p=0.04$, day × treatment: $F(6,66)=2.86$, $p=0.02$) was significantly reduced in rats with a warm plate as compared to rats without a plate. The largest difference in total RWA between rats with a warm plate and rats without a plate appeared on days 3 and 4. By analyzing average RWA at these days in 4 h bins, it was found that the reduced activity in rats with a warm plate was most prominent during the light phase (Fig. 3).

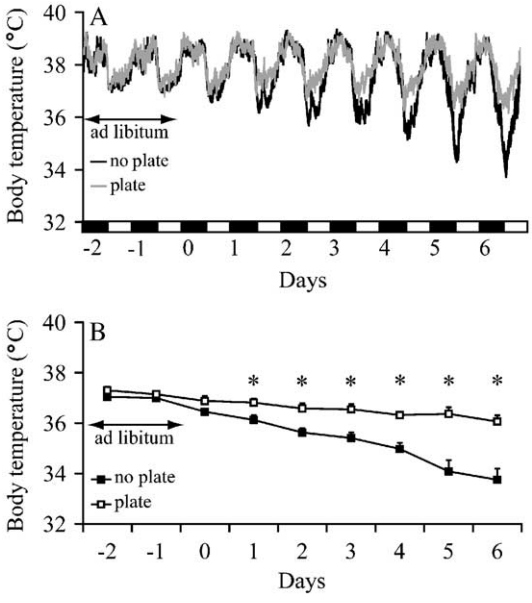

Fig. 2. Body temperature in ad libitum fed running rats and activity-based anorexia (ABA) rats with or without a warm plate. A. Daily body temperature during ad libitum feeding and following scheduled feeding in rats with (grey, $n=7$) or without (black, $n=6$) a warm plate. B. Basal body temperature (measured during inactivity in early light phase) during ad libitum feeding and following scheduled feeding in rats with (open squares, $n=7$) or without a plate (closed squares, $n=6$). Repeated measurements followed by T-test, $p\leq0.05$. *Significantly different from without a plate.

Table 2
Characteristics of activity-based anorexia (ABA) rats without a plate ($n=6$) and with a warm plate ($n=7$) in their cages

Treatment	Rats without a plate	Rats with a warm plate
Final body weight (% of initial body weight)	75.8±2.4	81.8±1.6*
Cumulative food intake (days 1–6) (g)	40.3±2.6	36.3±5.1
Adipose tissue (oviduct/ovaries) (mg)	626.2±207.8	685.8±111.2
Interscapular brown adipose tissue (mg)	85.4±4.6	127.8±17.7*
UCP1 gene expression (Rn) #	2.04±0.85	0.62±0.27
Leptin gene expression (Rn) #	0.09±0.05	0.05±0.05
Plasma leptin (ng/ml) $	n.d.	n.d.

Expression levels of uncoupling protein 1 (UCP1) and leptin were measured by quantitative PCR and were calculated as normalized ratio relative to a calibrator sample (Rn). $ Plasma leptin levels were analyzed by radioimmunoassay. T-test $p\leq0.05$. *Significantly different from without a plate. n.d.=not detectable.

3.4. Effects on food intake and body weight

The presence of a warm plate in the cage did not influence food intake (no plate: 21.0±0.9 g, warm plate: 21.5±4.4) ($t(11)=-0.26$, n.s.) or body weight (no plate: 240.8±4.4 g, warm plate: 248.9±4.0) ($t(11)=-1.39$, n.s.) during ad libitum feeding.

Statistical analysis revealed no effect of the presence of the warm plate on food intake during ABA (day: $F(5,50)=31.64$, n.s., day × treatment: $F(5,50)=1.29$, n.s.). Cumulative food intake was also not significantly different between the two groups ($t(10)=1.20$, n.s.) (Table 2). During ABA, a significant effect of treatment over time on relative body weight was observed (day: $F(6,66)=118.22$, $p<0.001$, day × treatment: $F(6,66)=3.99$, $p=0.05$). ABA rats with a warm plate showed a reduced body weight loss ($t(11)=1.86$, $p=0.04$) as compared to rats without a plate, whereas the amount of (isolated) white adipose tissue (WAT) was not significantly different between the two groups ($t(11)=-1.28$, n.s.). Plasma leptin levels were below detection limits in both groups. Furthermore, qPCR analysis revealed that expression levels of leptin mRNA in WAT were not different in ABA rats with or without a warm plate ($t(10)=0.51$, n.s.).

3.5. Association between body temperature and RWA

Above it was shown that body temperature was significantly reduced during the light phase, while unaffected during the dark phase in ABA rats without plates (Fig. 2). Furthermore it was shown that, in particular during the light phase, RWA was reduced by the presence of a warm plate (Fig. 3). In order to investigate whether a low body temperature elicited light phase RWA on days 3 and 4 (when considerable hypothermia and hyperactivity were observed), correlation analysis was performed.

Body temperature of ABA rats without a plate on days 3 and 4 was negatively correlated with light phase RWA on days 3 and 4 (Table 3). In addition, relative body weight was

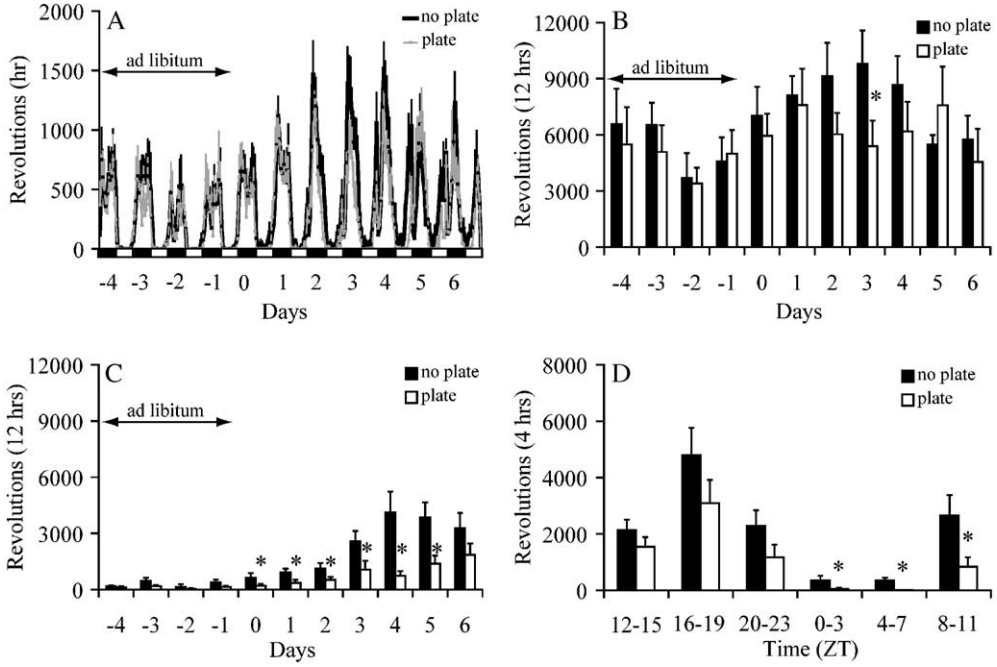

Fig. 3. Running wheel activity (RWA) in ad libitum fed rats and activity-based anorexia (ABA) rats with or without a warm plate. A. Daily RWA during ad libitum feeding and following scheduled feeding in rats with (grey, $n=7$) or without (black, $n=6$) a warm plate. B. RWA in the dark phase during ad libitum feeding and following scheduled feeding in rats with (white, $n=7$) or without (black, $n=6$) a warm plate. C. RWA in the light phase during ad libitum feeding and following scheduled feeding in rats with (white, $n=7$) or without (black, $n=6$) a warm plate. D. RWA analyzed in 4 h bins during days 3 and 4 in ABA rats with (white, $n=7$) or without (black, $n=6$) a warm plate. Repeated measurements followed by T-tests, $p \leq 0.05$. *Significantly different from without a plate.

also correlated with light phase RWA on these days. By analysis of activity (running wheel activity and locomotor activity) patterns and body temperature plots, it was found that minima in body temperature were not always immediately followed by bouts of running and that bouts of running were not always preceded by a low body temperature (Fig. 4), suggesting that hypothermia does not (at least not immediately) result in RWA. Though, it was observed that body temperature increased immediately following wheel running.

In rats with a warm plate, body temperature and relative body weight were not correlated with light phase RWA during the food restriction period.

Table 3
Correlation coefficients between basal body temperature/relative body weight and running wheel activity (RWA) during the light phase

		Light RWA day 3	Light RWA day 4
Basal body temperature versus RWA in the light phase			
No plate	Basal temperature #	$r=-0.82$	$r=-0.93$
		$p=0.04$*	$p<0.01$*
Warm plate	Basal temperature #	$r=-0.05$	$r=0.51$
		$p=0.93$	$p=0.25$
Relative body weight versus RWA in the light phase			
No plate	Rel. body weight $	$r=-0.81$	$r=-0.81$
		$p=0.05$*	$p=0.05$*
Warm plate	Rel. body weight $	$r=-0.56$	$r=-0.54$
		$p=0.19$	$p=0.21$

Basal body temperature was measured at the beginning of the light phase (ZT0–ZT3), days 3 and 4. $ Relative body weight was measured at ZT11 (days 2 and 3). Pearson correlation analysis, *$p \leq 0.05$.

4. Discussion

Here it was shown that by giving ABA rats voluntary access to a warm plate, hypothermia was prevented and hyperactivity during the light phase as well as body weight loss were reduced as compared to ABA rats without access to a plate. Rats with a warm plate had a higher BAT mass after exposure to ABA than rats without a plate, and tended to have decreased expression levels of UCP1 in BAT. Thus, reduced thermogenesis and reduced RWA prevented extreme body weight loss in rats with a warm plate.

In the present study, a warm plate was introduced into the cage, thereby creating a local heated place in the cage without influencing ambient temperature. Since the warm plate covered only a small area of the cage, rats could voluntarily make use of the warm plate. By video-monitoring (during the light phase), it was observed that rats made extensive use of the warm plate during ABA (days 3 and 4, but also on later days; data not shown), but had no preference for the plate during ad libitum feeding conditions (days-2 and -1, following a habituation period) (Fig. 1). Importantly, wheel running, body temperature, body weight and food intake were not influenced by the presence of a warm plate during ad libitum feeding, implying that the effects obtained were specific for ABA. As compared to RWA in the light period, RWA in the dark period was only modestly reduced by the presence of a warm plate. This suggests that during the normal active period possibly rewarding properties of running or foraging

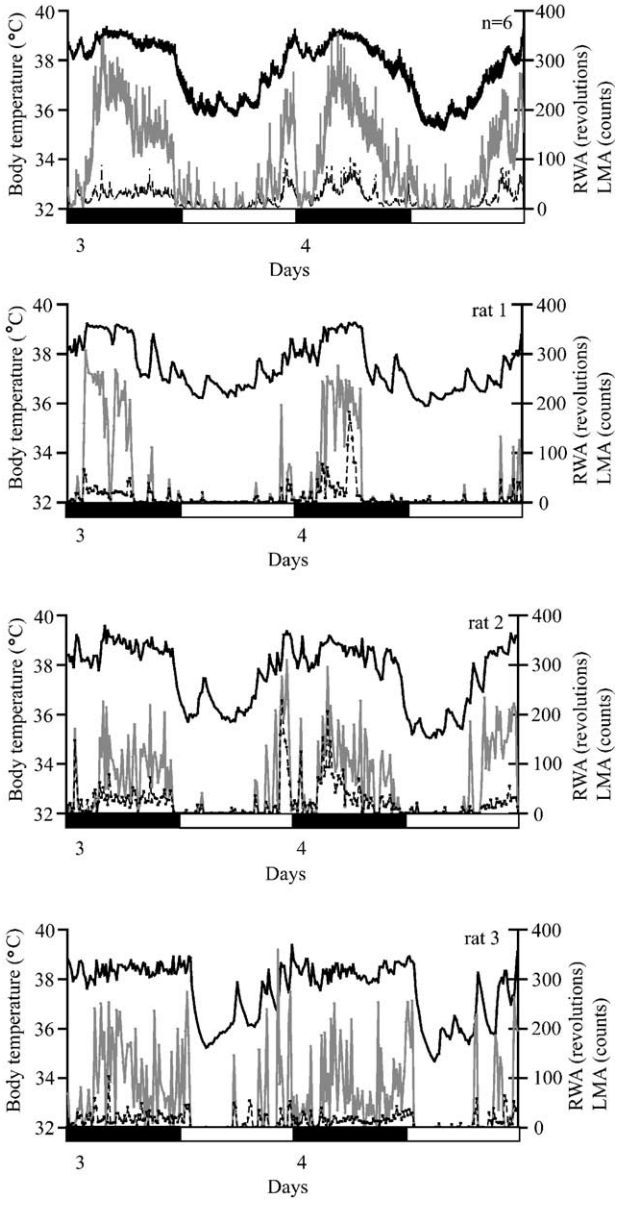

Fig. 4. Running wheel activity (RWA), locomotor activity (LMA) and body temperature in activity-based anorexia rats without a warm plate. RWA (grey line), LMA (black dashed) and body temperature (black) during days 3 and 4 of all rats ($n=6$) without a warm plate and three individual rats are depicted. Note that RWA (revolutions) and LMA (counts) are both plotted on the right *Y*-axis, using the same scale but different units. Data was analyzed in bins of 10 min.

behavior are more important for eliciting RWA than thermoregulatory processes.

Previously it was shown that rats are more motivated to bar press for heat during food restriction, resulting in reduced activity levels [15], which corresponds with our results. In addition, it was shown before that the likelihood and length of survival increases when ABA rats are heated with a lamp just before becoming moribund [16].

Basal body temperature was inversely correlated with RWA on days 3 and 4 when activity reached its highest levels. However, periods of light phase RWA were not immediately preceded by periods of hypothermia. Thus, no evidence was found for the hypothesis that decreases in body temperature directly trigger RWA in ABA rats. Therefore the relationship found between body temperature and light phase RWA probably has a longer time frame. It can also not be excluded that other factors, like circadian rhythmicity in body temperature and RWA, compromise the relationship between body temperature and RWA. Our data correspond with earlier reports showing that a decreased body temperature does not directly stimulate wheel running. Wheel running seems to be a better predictor of a rise in body temperature, than low body temperature is of a rise in wheel running [16], which was confirmed in this study (Fig. 4).

The relation between (environmental) temperature and RWA might also be explained as a primary effect of body weight loss. During ABA, body temperature decreases, and the body needs substantial energy to maintain temperature homeostasis. Thermogenesis results in a fast exhaustion of energy stores and a further decrease in body insulation and body weight loss. This body weight loss itself might also trigger hyperactivity. Indeed, it was hypothesized before that a decrease in plasma leptin levels associated with body weight loss might trigger hyperactivity [8]. In the present experiment it was observed that body weight loss was correlated with RWA in rats without a plate and that the presence of a warm plate reduced body weight loss. However, although body weight loss was significantly affected by access to a warm plate, plasma leptin levels were still undetectable and no differences in leptin expression levels in WAT were observed. This suggests that heat treatment in ABA overrules the putative role of decreased leptin levels in triggering hyperactivity [8].

It was shown before that rats housed in cages with running wheels reduce 1 h food intake as compared to 1 h-fed rats without running wheels on the final day of ABA [17]. While hypothermia, RWA and body weight loss were significantly reduced by presence of a warm plate, cumulative food intake (as well as food intake per day) was not significantly changed by the presence of a warm plate. Thus although a reduced 1 h food intake in ABA rats can be explained by prior excessive exercising [18], additional factors (like physical inability) might also result in hypophagia in ABA rats. Still, the absence of increased food intake in rats with a warm plate was surprising, but this might be explained by Brobeck's law, which states that rats eat to get warm and stop eating to prevent hyperthermia [19].

Anorexia nervosa patients suffer from extreme dieting and hyperactivity. Reduced fat mass results in decreased body insulation and impairment of thermoregulatory homeostasis. The effect of heat treatment in anorexia nervosa therapy has hardly been explored. Although reductions of anxiety, food refusal and hyperactivity were found following heat treatment in three anorexic patients [20], a randomized controlled trial on heat treatment (by heated vests) in anorexia nervosa patients did not show

significant effects on BMI [21]. Interestingly, heat treatment was recently introduced in the Swedish Mandometer treatment program [22].

In summary, it was shown that the presence of a warm plate prevented hypothermia and reduced hyperactivity and body weight loss in ABA rats. This suggests that rats prefer to prevent hypothermia passively by choosing a heated resource than actively regulating body temperature by hyperactivity. Extrapolating from these preclinical findings, it can be suggested that heat treatment in hypothermic anorexia nervosa patients might be beneficial as well; therefore these animal data urge for more clinical studies on heat treatment in anorexia nervosa patients.

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