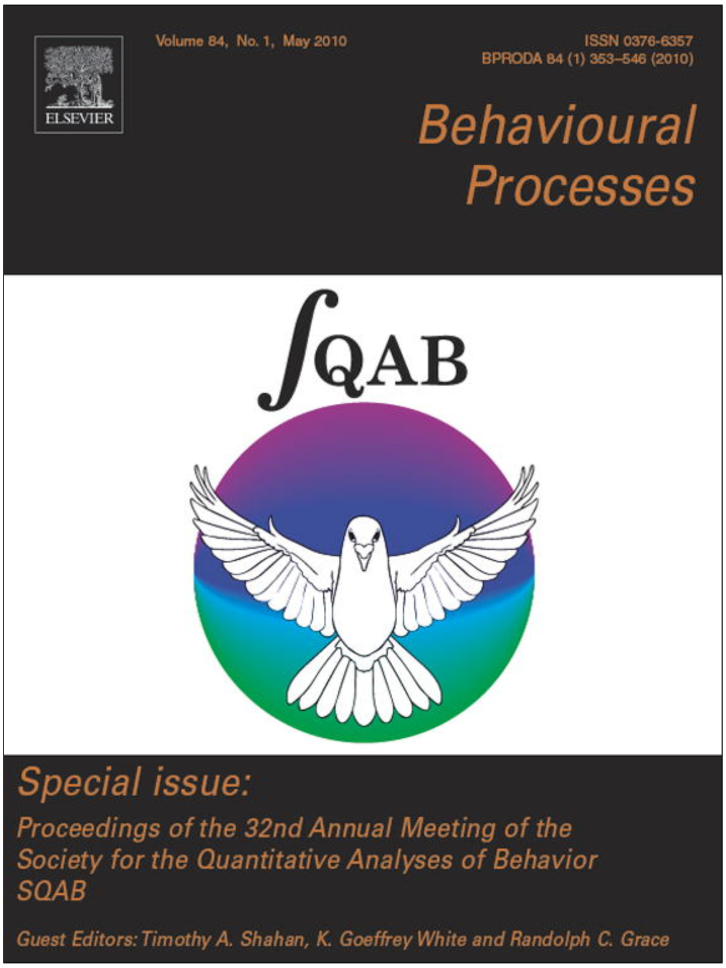
Volume 84, No. 1, May 2010
ISSN 0376-6357
BPRODA 84 (1) 353–546 (2010)
ELSEVIER
Behavioural Processes
∫QAB
Special issue:
Proceedings of the 32nd Annual Meeting of the Society for the Quantitative Analyses of Behavior SQAB
Guest Editors: Timothy A. Shahan, K. Goeffrey White and Randolph C. Grace

Behavioural Processes 84 (2010) 541–545

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

The effects of food presentation at regular or irregular times on the development of activity-based anorexia in rats

Ángeles Pérez-Padilla[a,*], Paula Magalhães[b], Ricardo Pellón[a]

[a] Animal Behavior Lab, Departamento de Psicología Básica I, Facultad de Psicología, Universidad Nacional de Educación a Distancia, Madrid, Spain
[b] Animal Learning and Behavior Lab, Instituto de Educação e Psicologia, Universidade do Minho, Braga, Portugal

ARTICLE INFO

Article history:
Received 21 September 2009
Received in revised form 7 February 2010
Accepted 8 February 2010

Keywords:
Activity-based anorexia
Regular periods of food
Irregular periods of food
Anticipatory running
Rats

ABSTRACT

Activity-based anorexia occurs when food availability is restricted to 1 h of the day and a wheel is freely available to the rest of the time. Under such conditions rats run excessively and stop eating even during periods in which food is available. A defining characteristic of the excessive activity is that there is a peak of running in the anticipation of food availability. The present study was designed to test whether the occurrence of the food period at different times of the light phase of the light–dark cycle (from 08:00 to 20:00 h) could impede or postpone the normal development of activity anorexia. We compared the effect of presenting the food at a fixed time of the light period with presenting it on a variable schedule. Far from impeding or postponing the development of activity-based anorexia, presenting food at irregular times resulted in a pronounced body-weight loss, a low food intake and an increase in locomotor activity. Animals ran excessively, with a peak at the start of the dark period, and again when lights were turned on in the experimental room (running in the anticipation of food). Both fixed and variable schedules of food availability resulted in the development of activity-based anorexia in rats.

1. Introduction

Hall and Hanford (1954) found that when rats were placed on a daily 23-h food deprivation schedule, 1-h feeding and free access to water, their activity was increased in a negatively accelerated fashion with the number of days of restricted feeding. Such increases in activity were accompanied by animals limiting their intake of food and lost weight quite rapidly, with the result that nearly 30% of their experimental animals died. This finding contrasted with the relatively constant level of activity and food intake of control animals with an *ad libitum* feeding schedule. Routtenberg and Kuznesof (1967) and Routtenberg (1968) called the phenomenon as self-starvation; later it was named activity–stress paradigm (Paré and Houser, 1973; Paré, 1975) and more recently as activity-based anorexia (Epling et al., 1983).

The development of activity-based anorexia requires the simultaneous presence of two conditions: restricted access to food and access to an activity wheel. Kanarek and Collier (1983) proposed that activity-based anorexia is due to a failure to adapt to the restricted feeding schedule, thus producing a reduction in food consumption and in body-weight (see also Dwyer and Boakes, 1997). According to this view, activity and food consumption have an indirect relation mediated by the failure of adaptation to the feeding schedule. An alternative position rests on the idea that activity acquires reinforcing properties, thus interfering with food intake directly (Epling and Pierce, 1988). It has been shown that moderate activity reduces the reinforcing effectiveness of food and that food deprivation increases the reinforcing properties of running. Furthermore, activity can also produce an increase in the sense of satiation, for smaller meals are more frequently ingested under conditions of high activity (Pierce et al., 1986).

Rats exposed to restricted access to food provided at a fixed time of the day, show robust anticipatory behaviors such as wheel running and lever pressing, beginning 1–3 h before mealtime (Bolles and Stokes, 1965; Mistlberger, 1994). Food-anticipatory activities arise if meal onset intervals are in the circadian range, approximately between 22 and 31 h (Stephan, 1981, 1992). Wheel running activity is controlled both by an external diurnal cues and by a biological clock with a natural 24-h period, but not by stimuli arising from food deprivation *per se*. In addition to food-anticipatory running, rats also show a peak of activity during the early hours of the dark part of the circadian cycle, but rarely run during the mornings or immediately after eating (Bolles and de Lorge, 1962; Bolles and Stokes, 1965). The running of rats during 24-h cycle is therefore related both to the time of the day and to the time of feeding. When Bolles and de Lorge (1962) tested rats on non-circadian cycles of 19 and 29 h of 1-h feeding they observed no food-anticipatory running, with 29-h rats showing activity cycles of 23–24 h.

* Corresponding author at: UNED, Psicologia Basica I, Juan del Rosal 10, Ciudad Universitaria, Madrid 28040, Spain. Tel.: +34 915082327; fax: +34 915082327.
E-mail address: aperezp@pas.uned.es (Á. Pérez-Padilla).

0376-6357/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.beproc.2010.02.007

Previous experiments in our laboratory have replicated the phenomenon of activity-based anorexia using the standard procedure of 60-min daily access to food and free access to a running wheel for the remaining 23 h (Cano et al., 2006; Gutiérrez and Pellón, 2008). The present study was designed to compare the effect on body-weight, food intake and running of restricted food presentations (1 h) over fixed versus variable periods of time during the light period of the 24-h cycle. A 2 × 2 factorial design was used for the test procedure: one factor was the type of feeding schedule – fixed versus variable – and the other was the presence or absence of a running wheel—active versus inactive. We examined whether the occurrence of food periods at unpredictable times would have an effect on anticipatory running that would affect the development of activity-based anorexia. We also examined the relative contributions of the failure of adaptation to the feeding regime and the interference of eating by running in the development of activity-based anorexia. If body-weight loss was the result of a lack of adaptation to the feeding schedule, the extent of the loss should be even greater when feeding periods are unpredictable, independently of whether a running wheel is provided. Alternatively, if body-weight loss was a direct result of the provision of the running wheel, body-weight loss should occur when the running wheel is provided, independently of whether the feeding periods are regular or irregular.

2. Method

2.1. Subjects

Thirty-two experimentally naïve female Wistar rats obtained from Charles River Laboratories (Lyon, France) were used. All animals were monitored and fed daily, and at 60 days of age began the experiment. They were individually housed in comfortable home cages and placed in a room with rigorously controlled environmental conditions (ambient temperature 21 °C, 60% relative humidity, and 8:00 a.m./8:00 p.m. light/dark cycle). Mean weight at the beginning of the experiment was 221.32 g (range 189–257 g). Animals were weighed daily at the start of the food period of each experimental day. Water was freely available to all animals. All animal care procedures were in accordance with the European Communities Council Directive 86/609/EEC and the Spanish Royal Decree 223/1998 for minimizing stress and discomfort in animals.

2.2. Apparatus

The experimental animals were housed in individual cages with attached running wheels. Eight translucent acrylic boxes of 21 cm × 45 cm × 24 cm were used. An activity wheel was situated on the right side of each box and measured 9 cm wide and 34 cm diameter (MED Associates Inc., Georgia, VT, USA). The running wheel was equipped with an operated brake. Finally, a water bottle was inserted on the left side of the ceiling grid of each box, thus being permanently accessible to the animals. Just behind the water bottle there was a concave area where food could be placed.

The control animals were housed in individual translucent acrylic cages of 18 cm × 32.5 cm × 20.5 cm. Control animals had constant access to water in the same way as experimental rats.

Programming and recording equipment (MED-PC for Windows software package) was located in a room separated from the housing room. Wheel turns and licks to water bottles were automatically monitored using a computer and stored at 15 min intervals for each subject.

2.3. Procedure

Two days after the subjects were moved to individual cages, the experimental sessions started. All animals were weighed daily at the beginning of the food period. Food availability was restricted to 1 h daily during the light period (from 08:00 to 20:00 h) of the light–dark cycle. Two types of procedures were used: sixteen animals received food at a fixed time every day from 13:00 to 14:00 h [an analogous to a fixed time (FT) 23-h food schedule], and the remaining sixteen animals were fed at a determined variable time each day from 9:00 to 10:00, 12:00 to 13:00, 15:00 to 16:00 or 18:00 to 19:00 h [an analogous to a variable time (VT) 23-h food schedule]. In both procedures, half of the animals (8 + 8) were housed individually in cages containing a running wheel (Wheel Groups, WG), and the other half (8 + 8) were housed individually in cages without running wheels (Cage Groups, CG). During the feeding period the brake was activated for the WG rats so that running could not compete with eating, but the rest of the time animals had free access to the running wheel. Water was continually and freely available to all animals, during both the feeding and the running periods.

Food and water consumption, wheel turns and body-weight were measured for each rat each day. An animal was removed from the experiment if in two consecutive days its weight dropped below 75% of its free-feeding value (Dwyer and Boakes, 1997). The experiment finished when the first rat had to be removed from the procedure, and this was done in order to have an equal number of animals in each group each session for later statistical analysis. When the experiment stopped, all animals were given free access to food and water, and experimental rats were transferred to cages without running wheels.

Results of percentage body-weight and food intake were subjected to three-factor analysis of variance (ANOVA) with two between-groups factors (Schedule and Wheel) and one within-subject factor (Sessions). Results of rate of running were subjected to a two-factor ANOVA with a between-subjects factor (Wheel) and a within-subject factor (Sessions). When necessary, post hoc comparisons were calculated by the Newman–Keuls test. All analyses were performed using the Statistica 5.0 package and the SPSS 17.0 package. The significance level was set at a minimum $p < 0.05$.

3. Results

Activity-based anorexia developed when animals simultaneously had access to a running wheel and were exposed to food restriction, regardless of whether food was presented at regular or irregular times within the light period of a 24-h light–dark cycle. Fig. 1 (top panel) shows the effect of wheel availability and feeding time procedure on body-weight as a percentage of weight at the beginning of the experiment. Percentage body-weights at the end of the experiment were significantly lower in the wheel groups (filled symbols) than in their corresponding cage groups (open symbols). In general, wheel availability incremented the reduction of body-weights in comparison to the control conditions, and the VT procedure resulted in a higher percentage of body-weight loss than the FT procedure independently of the accessibility to the running wheel. The ANOVA resulted in all principal effects: Wheel [$F(1,28) = 31.46$, $p < 0.001$], Schedule [$F(1,28) = 26.60$, $p < 0.001$] and Sessions [$F(9,252) = 617.69$, $p < 0.001$], as well as the Wheel × Sessions interaction [$F(9,252) = 25.78$, $p < 0.001$] and the Schedule × Sessions interaction [$F(9,252) = 13.54$, $p < 0.001$]. The Wheel × Schedule and the Wheel × Schedule × Sessions interactions were not statistically significant ($p > 0.05$).

Fig. 1 also shows the food intake (bottom panel) during the 1-h period of food access. The combined presence of the wheel and the feeding schedule decreased the amount of food intake in comparison to control conditions, especially under the FT procedure. Total food consumption was significantly greater in the caged groups: Wheel effect [$F(1,28) = 9.18$, $p = 0.005$], and the greatest intake at the end of experiment (about 9 g) was in the control condition during the FT procedure. These animals presented the lowest

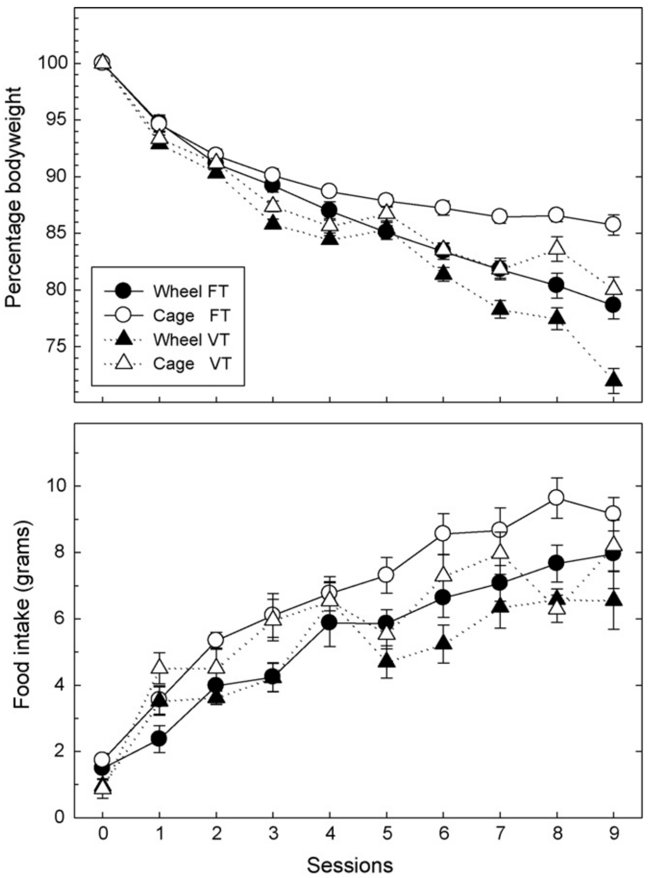

Fig. 1. Mean body-weight (±SEM) for the four groups in each session, measured as body-weight as a percentage of the initial weight when the animals started the experimental procedures (top panel), and mean daily (±SEM) food intake during the 1-h period of food access (bottom panel). *Circles* correspond to the groups exposed to the FT 23-h food schedule. *Triangles* correspond to the groups exposed to the VT 23-h food schedule. *Filled symbols* correspond to the animals with access to running wheels. *Open symbols* correspond to the animals without wheel access.

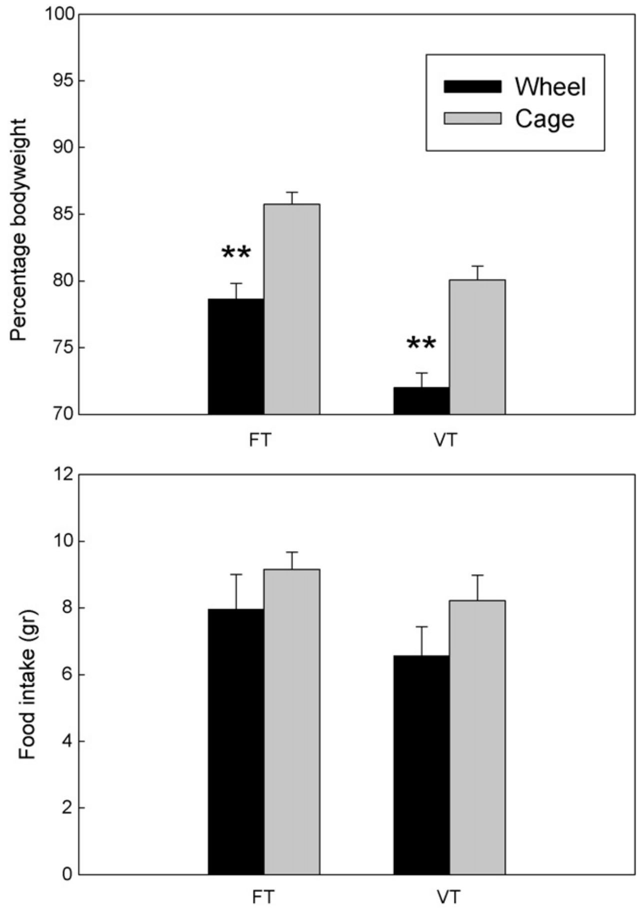

Fig. 2. Mean body-weight (±SEM) for the four groups in the last session, measured as percentage of body-weight with respect to the initial weight when the animals started the experimental procedures (top panel), and mean daily (±SEM) food intake during the 1-h period of food access (bottom panel). *Black bars* correspond to the groups with access to running wheels. *Grey bars* correspond to the groups without wheel access.

percentage of body-weight loss (only about 15%) during the last days of the experiment (see top panel). The wheel group with the VT procedure, on the contrary, ate the least food (about 6 g) and showed the greatest percentage of body-weight loss, a reduction of about 30% (see top panel). The ANOVA also resulted in a Sessions effect [$F(9,252) = 104.74$, $p < 0.001$], showing a trend for more food consumption as sessions progressed, as well as Wheel × Sessions [$F(9,252) = 2.04$, $p < 0.05$] and Schedule × Sessions [$F(9,252) = 5.05$, $p < 0.001$] interactions. There was not a principal Schedule effect and, as with the percentage body-weight, Wheel × Schedule and Wheel × Schedule × Sessions interactions were also not statistically significant ($p > 0.05$).

Fig. 2 shows data from the last session (Session 9). The presence of the wheel reduced body-weight to a similar degree for FT groups [$F(1,14) = 22.92$, $p < 0.001$] and VT groups [$F(1,14) = 27.40$, $p < 0.001$]. Food intake was also reduced similarly for the FT and VT groups as a result of the presence of the wheel (bottom panel), but in this case food consumption was not significantly lower in the wheel than in the cage conditions ($p > 0.05$). Body-weight reduction and food intake were lower for the VT conditions than for the FT conditions.

Fig. 3 shows the rate of running activity measured in wheel turns per hour for the groups with access to the running wheels in each daily session. Exposure to the activity-based anorexia procedures resulted in significant increases of running on the wheels as sessions progressed [$F(8,112) = 25.25$, $p < 0.001$]. Increases in activity were more pronounced under the VT than under the FT procedure

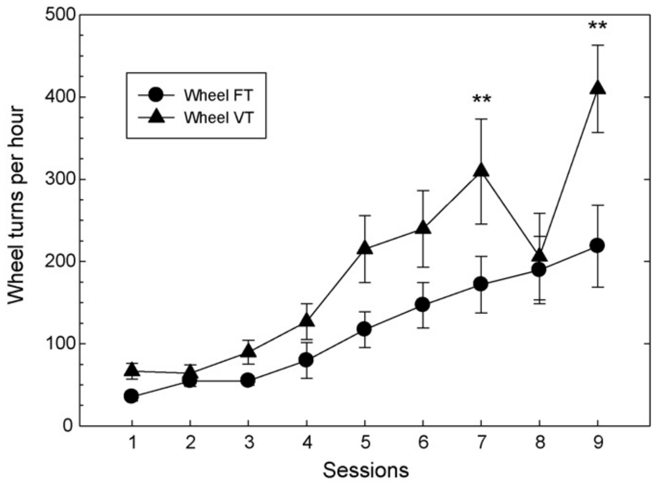

Fig. 3. Mean wheel turns per hour (±SEM) for the two groups with access to the running wheels in each daily session. *Circles* correspond to the group exposed to the FT 23-h food schedule. *Triangles* correspond to the group exposed to the VT 23 h schedule.

[$F(1,14) = 4.33$, $p = 0.05$]. Statistical analyses showed an interaction between Schedule and Sessions [$F(8,112) = 2.91$, $p < 0.005$]. Post hoc analysis confirmed that on Sessions 7 and 9 activity rate was higher on VT than on FT condition ($p < 0.005$).

Fig. 4 shows the temporal distribution of wheel running and licking the water spout within inter-meal episodes for the FT wheel group (upper panels) and the VT wheel group (bottom panels), just for the last five sessions of exposure to the activity-based anorexia procedure. Wheel turns and licks were recorded every 15 min across the 23-h period of activity. The black bars in the *x*-axis indicate the dark periods of the session, and the vertical dashed column indicates the moment of food presentation. For the FT group (upper panels), temporal distributions of both wheel running and licking were very similar, with a great degree of overlap. Both peaked at the start of the dark cycle (at about 20:00 h), and again in the anticipation of the occurrence of the food period—more or less 2 h before food was made available. In the case of drinking, there were also bouts of licking immediately after food was

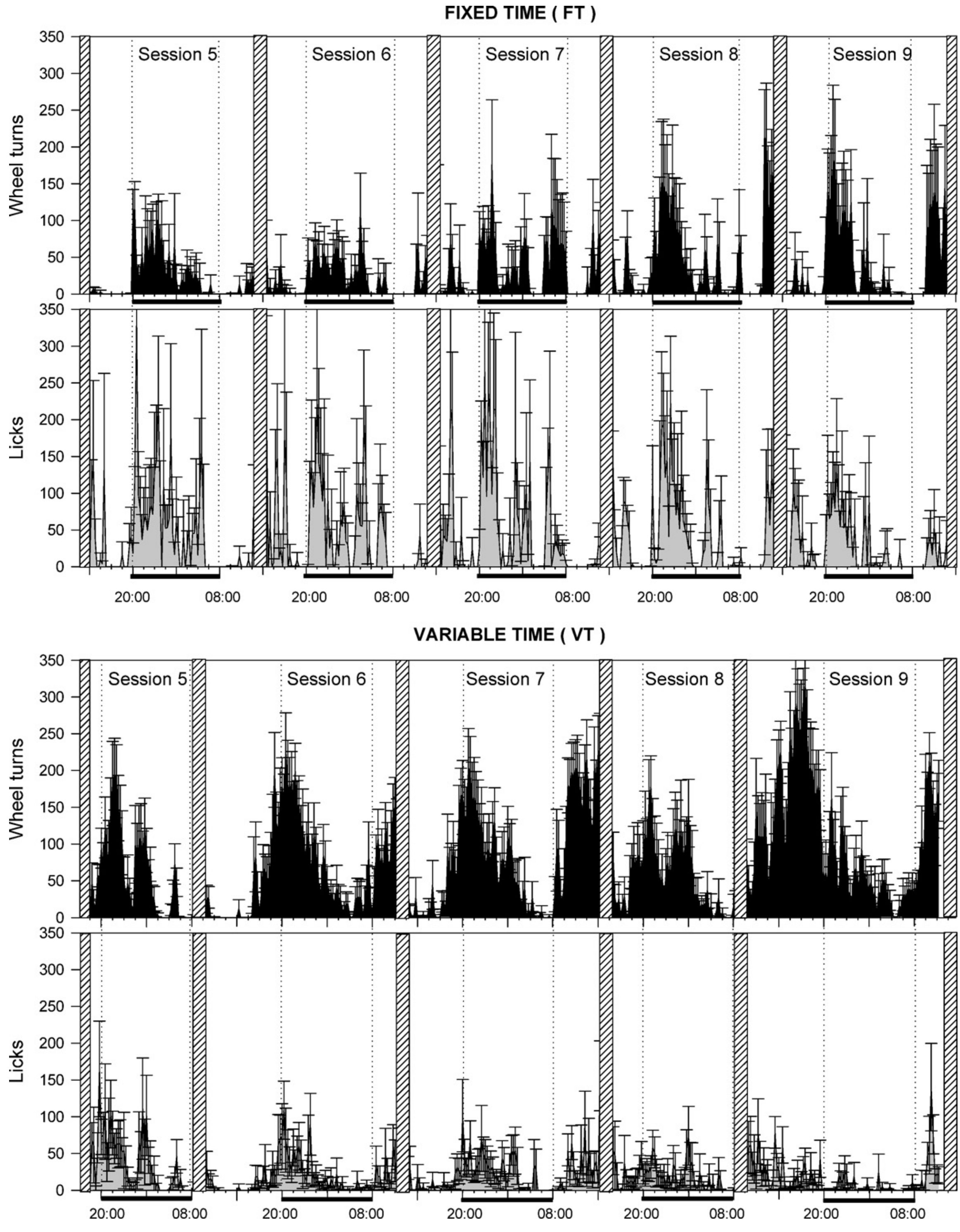

Fig. 4. Running and drinking patterns of wheel groups exposed either to FT 23-h of 1-h food presentation (top panel) or VT 23-h of 1-h food presentation (bottom panel), from Sessions 5 to 9. Each data point indicates the mean (±SEM) number of wheel turns and number of licks over successive 15-min periods. Lights were on from 08:00 to 20:00 h; dark periods are indicated by horizontal black bars in the *x*-axis. Vertical dashed columns indicate periods of food availability where wheels were locked.

removed, an observation also seen for wheel running but to a lesser degree.

A similar effect to the FT procedure was observed in the VT group when sessions were long enough, such as Sessions 6, 7 and 9 (lower panels). Note that sessions in the VT procedure could be extremely short or extremely long, thus influencing the opportunity to run or lick. Both running and licking showed similar temporal distributions, but here running was more accentuated than in the FT procedure and licking was less marked. Both curves peaked at the beginning of the dark period, and also at the beginning of the light period. It should be noted that VT animals could not anticipate the exact moment of the delivery of food, so running and licking started when lights were turned on. This pattern is clearly seen in Sessions 6, 7 and 9, and was absent in Sessions 5 and 8 because the food period started almost immediately after turning on the lights (explaining why animals in the Session 8 dropped their rate of running is shown in Fig. 3). In the VT condition there was not as much drinking (and running) in the period after food removal as in the FT condition.

4. Discussion

Activity-based anorexia occurs when food availability is restricted to 1 h a day and a running wheel if freely available the rest of the time. This combination leads to a gradual decline in body-weight and gradual increases in food intake (after a sharp drop) and running. Increases in food intake normally are not sufficient to compensate the energy expenditure of the excessive activity. In the present study, subjects developed activity-based anorexia regardless of whether food was presented at regular or irregular times. In comparison to no-wheel control groups, body-weight loss when a running wheel was available was no different when eating periods were regular as opposed to irregular. Furthermore, the irregular condition (with inter-meal episodes ranging between 14 and 32 h) was more effective than the regular condition (with fixed inter-meal episodes of 23 h) in speeding up the phenomenon (as shown in Figs. 1 and 3), and that was due to a general effect of the feeding schedule.

The experimental procedures employed in the present study resulted in a rate of wheel running that was always higher in the irregular than in the regular group (except for the second session at the start of the procedure and the eighth session for reasons explained above, as shown in Fig. 3). With respect to the pattern of excessive running, there was a peak of running in the anticipation of food availability and this was seen in both regular and irregular food procedures (see Fig. 4). In the case of irregular food presentation this was initiated when lights were turned on in the experimental room. All animals also showed an increase in activity during the dark part of the light–dark cycle. These results are in accordance with previous data from Dwyer and Boakes (1997) with a FT of 22.50-h food schedule and with food access during the light period.

Patterns of licking throughout the sessions showed a close relationship to patterns of running: peaks of activity during the dark and before and after food occurrence (see Fig. 4), and this adds to the literature showing correspondence between both the activities. Afonso and Eikelboom (2003) showed that running correlated positively with drinking in animals not submitted to food restriction and with stable running; here we demonstrate similar running–drinking relations under food restriction and during the process of acquisition.

According to the failure of adaptation view, activity-based anorexia should be primarily accompanied by a reduction in food intake (Kanarek and Collier, 1983; Dwyer and Boakes, 1997). According to the reinforcement view, anorexia is basically due to an increase in general activity (Epling and Pierce, 1988). Aspects of the present results seem to support both accounts. Loss in weight was affected by the schedule at which food was presented, being higher after aperiodic than periodic availability regardless of the possibility to exercise on the wheel, thus in accord with the failure of adaptation account. As shown in Fig. 1, there was a correspondence between food intake and percentage body-weight, with the group eating more having a less loss in weight (Cage FT) and the group eating less having the larger lost in body-weight (Wheel VT). Loss in weight was also affected by having access to the wheel, regardless of food being presented at regular or irregular times, thus in accord with a direct effect of running on weight loss (see top graph of Fig. 1). Body-weight loss was higher on the VT than the FT running conditions (see Fig. 3), thus further supporting a relationship between level of running and development of activity-based anorexia.

With the data at hand, we conclude that the occurrence of the food period at irregular times within the light period of the light–dark cycle did not impede or postpone the normal development of activity-based anorexia. On the contrary, it increased the running activity. In order to show the attenuation of anticipatory running by an irregular schedule of food it may be necessary to introduce food periods during both the light and the dark phases of the light–dark cycle, with the result that the development of activity-based anorexia may be affected.

Acknowledgements

Research and preparation of the manuscript was supported by the research grants SEJ2005-04633/PSIC and PSI2008-03660 from the Spanish Government. We thank Miguel Ángel Villajos for the help in maintaining the animals.

References

Afonso, V.M., Eikelboom, R., 2003. Relationship between wheel running, feeding, drinking, and body weight in male rats. Physiology and Behavior 80, 19–26.

Bolles, R.C., de Lorge, J., 1962. The rat's adjustment to a-diurnal feeding cycles. Journal of Comparative and Physiological Psychology 55, 760–762.

Bolles, R.C., Stokes, L.W., 1965. Rat's anticipation of diurnal and a-diurnal feeding. Journal of Comparative and Physiological Psychology 60, 290–294.

Cano, C., Gutiérrez, M., Pellón, R., 2006. Preexposición al programa de comida y desarrollo de anorexia basada en actividad en ratas. International Journal of Psychology and Psychological Therapy 6, 273–286.

Gutiérrez, M.T., Pellón, R., 2008. Ausencia de efecto del cambio de contexto de comida en el fenómeno de anorexia por actividad en ratas. Psicothema 20, 450–455.

Dwyer, D.M., Boakes, R.A., 1997. Activity-based anorexia in rats as failure to adapt to a feeding schedule. Behavioral Neuroscience 111, 195–205.

Epling, W.F., Pierce, W.D., 1988. Activity-based anorexia: a biobehavioral perspective. International Journal of Eating Disorders 7, 475–485.

Epling, W.F., Pierce, W.D., Stefan, L., 1983. A theory of activity-based anorexia. International Journal of Eating Disorders 3, 27–46.

Hall, J.F., Hanford, P.V., 1954. Activity as a function of a restricted feeding schedule. Journal of Comparative and Physiological Psychology 47, 362–363.

Kanarek, R.B., Collier, G.H., 1983. Self-starvation: a problem of overriding the satiety signal? Physiology and Behavior 30, 307–311.

Mistlberger, R.E., 1994. Circadian food-anticipatory activity: formal models and physiological mechanisms. Neuroscience and Biobehavioral Reviews 18, 171–195.

Paré, W.P., 1975. The influence of food consumption and running activity on the activity–stress ulcer in the rat. American Journal of Digestive Diseases 20, 262–273.

Paré, W.P., Houser, V.P., 1973. Activity and food restriction effects on gastric glandular lesions in the rat: the activity–stress ulcer. Bulletin of the Psychonomic Society 2, 213–214.

Pierce, W.D., Epling, W.F., Boer, D.P., 1986. Deprivation and satiation: the interrelations between food and wheel running. Journal of the Experimental Analysis of Behavior 46, 199–210.

Routtenberg, A., Kuznesof, A.W., 1967. Self-starvation of rats living in activity wheels on a restricted feeding schedule. Journal of Comparative and Physiological Psychology 64, 414–421.

Routtenberg, A., 1968. "Self-starvation" of rats living in activity wheels: adaptation effects. Journal of Comparative and Physiological Psychology 66, 234–238.

Stephan, F.K., 1981. Limits of entrainment to periodic feeding in rats with suprachiasmatic lesions. Journal of Comparative Physiology 143, 401–410.

Stephan, F.K., 1992. Resetting of a feeding-entrainable circadian clock in the rat. Physiology and Behavior 52, 985–995.