Food restriction-induced hyperactivity: Addiction or adaptation to famine?

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Summary Increased physical activity is present in 30–80% of anorexia nervosa patients. To explain the paradox of low food intake and excessive exercise in humans and other animals, it has been proposed that increased physical activity along with food restriction activates brain reward circuits and is addictive. Alternatively, the fleeing-famine hypothesis postulates that refusal of known scarce energy-low food sources and hyperactivity facilitate migration towards new habitats that potentially contain new energy-rich foodstuffs. The use of rewarding compounds that differ in energy density, such as the energy-free sweetener saccharin and the energy rich sucrose makes it possible to critically test the reward-addiction and fleeing-famine hypotheses. The aims of the present work were to study if sucrose and/or saccharin could attenuate food restriction-induced hyperactivity, weight loss, increased plasma corticosterone, and activation of brain structures involved in neuroendocrine control, energy balance, physical activity, and reward signaling in rats. Its major findings are that access to sucrose, but not to saccharin, attenuated food restriction-induced running wheel activity, weight loss, rises in plasma corticosterone, and expression of the cellular activation marker c-Fos in the paraventricular and arcuate hypothalamus and in the nucleus accumbens. These findings suggest that the energy-richness and easy availability of sucrose interrupted a fleeing-famine-like hyperactivity response. Since corticosterone mediates food restriction-induced wheel running (Duclos et al., 2009), we propose that the attenuating effect of sucrose consumption on plasma corticosterone plays a role in reduced wheel running and weight loss by lowering activation of the nucleus accumbens and arcuate hypothalamus in these animals.

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1. Introduction

Increased physical activity is present in 30–80% of anorexia nervosa patients (Davis et al., 1994; Klein et al., 2007) and is generally considered as a strategy to lose weight. However, food restriction by itself can lead to increased physical activity in anorexia nervosa (Holtkamp et al., 2004). In addition, several reports indicate that increased physical activity is linked to a compulsive component (Davis et al., 1995; Holtkamp et al., 2003) suggesting that it is not under cognitive control. The interactions between low food intake and excessive physical activity can be addressed in animals using behavioral paradigms, known as activity-based anorexia (Burden et al., 1993) and food restriction-induced hyperactivity (Broocks et al., 1990; Duclos et al., 2005). In these paradigms, rodents with free access to food display spontaneous wheel running that is covered by energy intake, whereas animals for which access to food is limited in amount or in time engage in excessive running that leads to denutrition and ultimately to death (Siegfried et al., 2003).

To explain the paradox of low food intake and excessive exercise in humans and other animals, it has been proposed that increased physical activity along with food restriction activates brain reward circuits (Bergh and Sodersten, 1996; Fladung et al., 2010). The observations that high scores on addiction scales in anorexia nervosa patients are related to excessive exercising (Davis and Claridge, 1998; Klein et al., 2004) corroborate this reward-addiction hypothesis. Since food restriction is known to increase drug reward (Carr, 2007), it may well be that it also increases the reward of physical activity.

Alternatively, the fleeing-famine hypothesis postulates that refusal of normal scarce energy-low or hard-to-assimilate food sources and hyperactivity facilitate migration towards new habitats that may contain new energy-rich or easily-assimilable foodstuffs (Guisinger, 2003). The occurrence of hyperactivity upon reduced energy availability in many animal species suggests that it represents an adaptive response. According to the fleeing-famine hypothesis, initial food restriction to lose weight in humans may activate brain structures underlying this phylogenetically old response and induce regular food refusal and hyperactivity, thus leading to further weight loss (Guisinger, 2003).

Free access to glucose attenuates food restriction-induced wheel running and weight loss in animals (Takeda et al., 2003). In addition, the reinforcement value of wheel running can be substituted to a certain extent by sucrose in food-restricted, but weight-stable, rats (Belke et al., 2006). Finally, limited access to a sweet and high-fat diet prevents food restriction-induced weight loss but without altering daily wheel running (Brown et al., 2008). These findings, however, can be explained both by the reward-addiction and by the fleeing-famine hypotheses, since glucose, sucrose and fat are both rewarding and energy-rich. Interestingly, wheel running decreases cocaine self-administration and vice versa (Cosgrove et al., 2002). Furthermore, the reinforcing value of cocaine can be substituted by the artificial energy-free sweetener saccharin (Lenoir et al., 2007). These observations led us to critically test the reward-addiction and fleeing-famine hypotheses by comparing the effects sucrose to those of saccharine on food restriction-induced wheel running.

The main aim of the present work was, therefore, to study if sucrose and/or saccharin could attenuate food restriction-induced hyperactivity and weight loss. Since we have previously shown that corticosterone mediates food restriction-induced hyperactivity (Duclos et al., 2009), we also measured its plasma concentrations and the cellular activation marker c-Fos in the paraventricular nucleus of the hypothalamus (PVH), known to control activity of the hypothalamus—pituitary—adrenal axis. Finally, we studied cellular activation in the nucleus accumbens and arcuate nucleus of the hypothalamus (ARH), two brain structures involved in reward signaling, physical activity and energy balance (Werme et al., 2002; Badman and Flier, 2005).

2. Methods

2.1. Animals

Experiments were conducted on 112 male Lewis rats (Charles River, L’Arbresle, France) known to be sensitive to food restriction-induced activity (Duclos et al., 2005). Four week-old animals were housed four per cage with ad libitum access to food (standard laboratory chow OA4; UAR, Villelmoison, France) and water at constant temperature (23–25 °C) and a 12 h light–dark cycle (lights on at 0700 h). They were left undisturbed for two weeks before being housed individually when their body weight reached 175–180 g. Experiments were conducted according to French and European recommendations on animal research (European Council Directive of 24 November 1986 (86/609/EEC)).

2.2. Experimental protocol

On day 1 of the experiment, after 14 days of habituation to the animal colony, rats were placed in individual cages and kept in a temperature (23–25 °C)- and light (lights on 0700–1900 h)-controlled room that housed all cages, running wheels and recording equipment necessary for the experiment. To address the effects of food restriction and running wheel activity, animals were housed individually and randomly assigned to one of the four experimental conditions: ad libitum access to food, but not to a running wheel (AL), ad libitum access to both food and a running wheel (ACT), food restriction in the absence of a running wheel (FR) and food restriction with access to a running wheel (FR-ACT). Rats in the FR-ACT group had access to the running wheel for 22.5 h during which they were food-deprived. During the remaining 1.5 h animals were locked out of the wheels and given free access to food starting at 1500 h. Animals in the FR group were given a daily food allowance corresponding to the meal of a previously assigned FR-ACT rat. The effects of sucrose or saccharin consumption were studied by providing half of the rats with a two-bottle choice consisting of plain water and 0.88 M sucrose (SUC) solution and the other half with plain water and 0.002 M saccharin (SAC) solution. Since saccharin is 300–500 times sweeter than sucrose (Wiet and Beyts, 1992) and the concentration of saccharin was 440-fold lower than that of sucrose, the saccharin and sucrose solutions used in the present study were comparably sweet. Thus, the experimental design included three independent variables each with two levels: (1) food intake: restricted or ad libitum
access, (2) activity: access or not to a running wheel, and (3) sweetened drinking water: *ad libitum* access to a sucrose or saccharin solution in addition to plain water. In a first study, a set of 62 animals were randomly assigned to the eight experimental groups and followed until day 6 (*n* = 7–9 rats per group). Throughout the experiment, rats were weighed at 1500 h.

When an animal in the FR-ACT group lost 25% of its initial body weight, usually around day 6 (Duclos et al., 2005), it was considered to have met the weight-loss criterion for FR-ACT and killed. Since the first study indicated that food-restricted rats significantly increased their sucrose consumption from day 6 onwards, another set of 50 rats were assigned to the same experimental groups, except those in the FR SAC and FR-ACT SAC groups that had lost 25% of their initial body weight on day 6, and followed until day 16 (*n* = 7–9 rats per group).

### 2.3. Plasma and tissue preparation

At 1500 h on day 6 or 16 after the start of the experiment, animals were removed from their cage and anesthetized with pentobarbital (60 mg/kg) in an adjacent room within 30 s of removal from their home cage. Intracardiac blood was collected in 10 ml polyethylene tubes containing 0.1 ml of 10% EDTA, and stored in ice until centrifugation. Then, the animals were fixed by intracardiac perfusion of 4% paraformaldehyde in 0.1 M PBS (pH 7.5 at 10 °C for 15 min) after a short rinse with 0.1 M PBS. The brain was removed from the skull, post-fixed for 4 h in the same fixative and cryoprotected in 30% sucrose in 0.1 M PBS for 48 h. Brain and plasma were frozen and stored at −80 °C until analysis. Adrenal glands were excised, trimmed of fat and weighed. The thymus gland was removed and weighed as an additional indicator of peripheral glucocorticoid status (Dallman et al., 1999). White adipose tissue (retroperitoneal, epididymal and subcutaneous inguinal) was dissected and weighed.

### 2.4. Corticosterone measurements

Plasma corticosterone was measured in duplicate after alcohol extraction by a competitive protein binding assay (Duclos et al., 2005) using rhesus monkey serum as the source of transcortin, [3H]corticosterone as the tracer, and dextrancoated charcoal to absorb the unbound fraction (sensitivity 4 ng/ml, specificity > 95%, inter assay coefficient of variation 8.0%).

### 2.5. c-Fos and FosB immunohistochemistry

Brains were cut, stocked and processed for immunohistochemistry as previously described (Konsman and Blomqvist, 2005). Commercially available antisera to c-Fos and FosB (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:1000 were used in a standard avidin–biotin–peroxidase protocol (Vectastain Elite, Vector Laboratories, Burlingame, CA; 1:1000) with nickel-enhanced diaminobenzidine as a chromogen. FosB was detected using a rabbit antiserum raised against a recombinant protein corresponding to the amino terminus of FosB of human origin (sc-7203). Because the truncated form ΔFosB lacks part of the FosB C-terminus, but is identical to FosB at the amino terminus, the FosB antiserum will detect both full-length FosB and ΔFosB.

Stained sections were examined with a microscope (Leica Microsystems, Wetzlar, Germany) and images were captured by a high-resolution CCD video camera image and fed into a personal computer as previously described (Konsman and Blomqvist, 2005). The number of c-Fos- and FosB-immunoreactive cells was measured in at least four sections of the dorsal nucleus accumbens containing both the shell and core territories (between bregma 0.45 and 1.25 mm) and previously shown to display increased expression of these cellular activation markers during cancer-associated increased energy expenditure and reduced food intake (Konsman and Blomqvist, 2005), in at least four sections of the PVH (between bregma 1.08 and −1.78 mm) and in at least three sections of the ARH (between bregma −2.0 and −3.25 mm). Since it was logistically impossible to process sections of over 60 animals in parallel and since we have previously shown that Fos expression in the ventral striatum and hypothalamus was low in *ad libitum*-fed sedentary rats and that food restriction in animals without access to a running wheel does not alter the number of c-Fos- and FosB-immunoreactive cells in these structures (Konsman and Blomqvist, 2005), experiments were performed on those animals that had access to a running wheel.

### 2.6. Statistical analysis

Body weight, food intake, intake of sweetened drinking water and wheel activity were expressed as means ± SEM and analyzed using a repeated measures analysis of variance (ANOVA). Thymus, adrenal and fat pad weights relative to body weight, serum corticosterone concentrations as well as the number of c-Fos- and FosB-immunoreactive cells were analyzed using ANOVA. In case of a significant interaction between factors, *a posteriori* comparisons were performed using Newman–Keuls tests.

### 3. Results

#### 3.1. Food-restricted rats with access to a running wheel prefer sucrose in the long run

An ANOVA on sweetened drinking water intake until day 6 showed significant main effects of and interactions between factors (Table 1). *Post hoc* analyses notably revealed that sweetened water intake until day 6 was higher in food-restricted animals consuming sucrose solution (FR SUC) compared to those having access to a saccharin solution (FR SAC; Table 1 and Figure 1A). An ANOVA on sucrose consumption until day 16 showed significant main effects of and interactions between factors (Supplementary Table S1). *Post hoc* analyses indicated that sucrose consumption compared to the first day of the experiment increased in food-restricted animals with access to a running wheel (FR-ACT SUC) from day 7 onwards (Supplementary Table S1 and Fig. S1A).

An ANOVA on the consumption of plain drinking water until day 6 revealed significant main effects of and interactions between factors (Table 1). *Post hoc* analyses showed that plain water intake was higher in food-restricted animals that had access to a running wheel and consumed saccharin...
<table>
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<th>Post hoc</th>
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<td>Sweet water intake</td>
<td>Food: $F_{1,104} = 5.69; p &lt; 0.05$</td>
<td>Food × wheel: $F_{1,104} = 26.7; p &lt; 0.0001$</td>
<td>FR SUC &gt; FR SAC D1—5;</td>
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<td></td>
<td>Wheel: $F_{1,104} = 172; p &lt; 0.0001$</td>
<td>Food × sweet: $F_{1,104} = 12.7; p &lt; 0.001$</td>
<td>$p &lt; 0.05$—0.01</td>
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<td></td>
<td>Sweet: $F_{1,104} = 42.2; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,104} = 23.1; p &lt; 0.0001$</td>
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<td></td>
<td>Day: $F_{4,416} = 9.27; p &lt; 0.0001$</td>
<td>Food × wheel × sweet: $F_{1,104} = 3.93; p = 0.05$</td>
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<td>Food × day: $F_{4,416} = 4.90; p &lt; 0.001$</td>
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<td>Sweet × day: $F_{4,416} = 5.12; p &lt; 0.001$</td>
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<td>Food: $F_{1,103} = 11.0; p &lt; 0.01$</td>
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<td>FR-ACT SAC D2—4 &gt; FR-ACT SAC D1;</td>
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<td>Wheel × day: $F_{4,412} = 3.32; p &lt; 0.05$</td>
<td>$p &lt; 0.05$—0.01</td>
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<td>Sweet: $F_{1,103} = 15.2; p &lt; 0.001$</td>
<td>Wheel × day: $F_{4,412} = 6.77; p &lt; 0.0001$</td>
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<td></td>
<td>Day: $F_{4,412} = 8.85; p &lt; 0.0001$</td>
<td>Food × wheel × sweet × day: $F_{3,312} = 2.59; p &lt; 0.05$</td>
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<tr>
<td>Wheel running: 24 h</td>
<td>Food: $F_{1,53} = 188; p &lt; 0.0001$</td>
<td>Food × sweet: $F_{1,53} = 5.28; p &lt; 0.05$</td>
<td>FR-ACT SUC &lt; ACT SUC D3—5;</td>
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<tr>
<td></td>
<td>Sweet: $F_{1,53} = 4.35; p &lt; 0.05$</td>
<td>Food × sweet × day: $F_{4,212} = 5.49; p &lt; 0.001$</td>
<td>$p &lt; 0.0001$—0.001</td>
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<td></td>
<td>Day: $F_{4,212} = 196; p &lt; 0.0001$</td>
<td>Food × sweet × day: $F_{4,212} = 4.15; p &lt; 0.05$</td>
<td>FR-ACT SUC &lt; FR-ACT SAC D4;</td>
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<td>Wheel × sweet × day: $F_{4,212} = 2.60; p &lt; 0.05$</td>
<td>$p &lt; 0.0001$—0.001</td>
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<tr>
<td></td>
<td></td>
<td>Day: $F_{4,212} = 24.0; p &lt; 0.0001$</td>
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<tr>
<td>Food intake</td>
<td>Food: $F_{1,104} = 4133; p &lt; 0.0001$</td>
<td>Food × wheel × sweet: $F_{1,104} = 51.6; p &lt; 0.0001$</td>
<td>AL SAC &gt; AL SUC; $p &lt; 0.01$—0.001</td>
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<td></td>
<td>Sweet: $F_{1,104} = 74.1; p &lt; 0.0001$</td>
<td>Food × day: $F_{3,312} = 2.73; p &lt; 0.05$</td>
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<td></td>
<td>Day: $F_{3,312} = 2.73; p &lt; 0.05$</td>
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<tr>
<td>Energy intake</td>
<td>Food: $F_{1,104} = 1215; p &lt; 0.0001$</td>
<td>Food × wheel: $F_{1,104} = 6.65; p &lt; 0.05$</td>
<td>AL &gt; FR; $p &lt; 0.001</td>
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<td></td>
<td>Wheel: $F_{1,104} = 161; p &lt; 0.0001$</td>
<td>Food × sweet: $F_{1,104} = 16.3; p &lt; 0.001$</td>
<td>AL SUC &lt; AL SAC; $p &lt; 0.01$—0.001</td>
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<td></td>
<td>Sweet: $F_{1,104} = 197; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,104} = 63.2; p &lt; 0.0001$</td>
<td>AL SUC &lt; ACT SUC; $p &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>Day: $F_{3,312} = 14.3; p &lt; 0.0001$</td>
<td>Wheel × day: $F_{3,312} = 3.74; p &lt; 0.05$</td>
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<tr>
<td></td>
<td></td>
<td>Sweet × day: $F_{3,312} = 5.14; p &lt; 0.001$</td>
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<tr>
<td>Body weight</td>
<td>Food: $F_{1,104} = 2935; p &lt; 0.0001$</td>
<td>Food × wheel × sweet: $F_{1,104} = 17.4; p &lt; 0.001$</td>
<td>AL SAC &gt; AL SUC D6; $p &lt; 0.001$</td>
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<tr>
<td></td>
<td>Wheel: $F_{1,104} = 87.1; p &lt; 0.0001$</td>
<td>Food × wheel × sweet × day: $F_{5,520} = 7.70; p &lt; 0.001$</td>
<td>FR-ACT SUC &lt; FR SUC D4—6; $p &lt; 0.05$—0.001</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,104} = 17.6; p &lt; 0.0001$</td>
<td>FR-ACT SUC &lt; FR SUC D6; $p &lt; 0.001$</td>
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<tr>
<td></td>
<td>Day: $F_{3,312} = 55.6; p &lt; 0.0001$</td>
<td>FR-ACT SUC &lt; FR-ACT SAC D6; $p &lt; 0.001$</td>
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</tbody>
</table>

Main effects of and interactions between food availability (Food), running wheel access (Wheel) and sweetening molecule (Sweet) on sweet and plain water intake, daily and preprandial wheel running, chow and energy intake, and body weight until day 6 as identified by repeated measures ANOVAs. Post hoc analyses indicate significant differences between groups and days (D) that were relevant to our hypotheses or unexpected. AL: rats with ad libitum access to food, but not to a running wheel; ACT: animals with ad libitum access to both food and a running wheel; FR: rats subject to food restriction in the absence of a running wheel; FR-ACT: animals subject to food restriction with access to a running wheel; SAC: freely available saccharin-sweetened water in addition to plain drinking water; SUC: freely available sucrose-sweetened water in addition to plain drinking water.
3.2. Food restriction-induced running wheel activity was higher in animals with access to saccharin compared to those having access to sucrose

An ANOVA on 24 h running wheel activity until day 6 indicated significant main effects of and interactions between factors (Table 1). Post hoc tests revealed that 24 h running wheel activity among animals that had access to saccharin was higher in those that were food-restricted (FR-ACT SAC) compared to those that had ad libitum access to food (ACT-SAC) on days 3, 4 and 5 of the experiment (Table 1 and Figure 2A). In a similar vein, these analyses showed that 24 h running wheel activity among animals that had access to sucrose was higher in those that were food-restricted (FR-ACT SUC) compared to those that had ad libitum access to food (ACT-SUC) on days 3, 4 and 5 of the experiment (Table 1 and Figure 2A). Finally, post hoc analyses indicated that on day 4 running wheel activity was higher in food-restricted rats with access to saccharin (FR-ACT SAC) compared to food-restricted animals consuming sucrose (FR-ACT SUC; Table 1 and Figure 2A). An ANOVA on 24 h running wheel activity of animals with access to sucrose until day 16 indicated significant main effects of and an interaction between factors (Supplementary Table S1). Post hoc tests showed that 24 h running wheel activity compared to the first day of the experiment was significantly higher in food-restricted animals from day 3 onwards (FR-ACT SUC; Supplementary Table S1 and Fig. S2A). It was also higher in ad libitum-fed rats, but only from day 8 onwards (ACT-SUC; Supplementary Table S1 and Fig. S2A).
Sucrose attenuates food restriction-induced hyperactivity and forebrain activation

Since food-restricted rats show a peak of wheel running in the diurnal period preceding the anticipated meal (from 1200 to 1500 h) (Duclos et al., 2005, 2009), we also studied preprandial activity. A repeated measures ANOVA on preprandial running wheel activity until day 6 showed significant main effects of and interactions between factors (Table 1). Post hoc tests revealed that preprandial running wheel activity among animals that had access to saccharin was higher in those that were food-restricted animals (FR-ACT SAC) compared to those that had ad libitum access to food (ACT-SAC) on days 3 and 4 of the experiment (Table 1 and Figure 2B). Similarly, these analyses showed that preprandial running wheel activity among animals that had access to sucrose was higher in those that were food-restricted animals (FR-ACT SUC) compared to those that had ad libitum access to food (ACT-SUC) on day 4 of the experiment (Table 1 and Figure 2B).

Finally, post hoc analyses indicated that on day 4 preprandial running wheel activity was higher in food-restricted rats with access to saccharin (FR-ACT SAC) compared to food-restricted animals consuming sucrose (FR-ACT SUC; Table 1 and Figure 2B). An ANOVA on preprandial running wheel activity of animals with access to sucrose until day 16 revealed no significant main effects or interactions (Supplementary Table S1).

3.3. Rats showing food restriction-induced hyperactivity increased their energy intake through sucrose consumption before they ate more chow

An ANOVA on food intake until day 6 revealed significant main effects of and interactions between factors (Table 1). Post hoc analyses indicated that food intake was higher in ad libitum-fed rats consuming saccharin (AL SAC) solution compared to those having access to a sucrose solution (AL SUC; Table 1 and Figure 3A). An ANOVA on food intake in animals with access to sucrose until day 16 identified significant main effects of and interactions between factors (Supplementary Table S1). Post hoc analyses showed that food intake in animals with limited access to food was significantly higher from day 10 onwards compared to the first day of the experiment regardless of whether they had access to a running wheel or not (Supplementary Table S1 and Fig. S3A).

An ANOVA on total energy intake (food (2.9 kcal/g) plus sucrose (1.2 kcal/ml)) until day 6 revealed significant main effects of and interactions between factors (Table 1). Post hoc analyses indicated that energy intake was higher in ad libitum-fed rats than in food-restricted animals (Table 1 and Figure 3B). In addition, these analyses revealed that energy intake was higher in ad libitum-fed rats consuming saccharin (AL SAC) solution compared to those having access to a sucrose solution (AL SUC; Table 1 and Figure 3B). Finally, post hoc analyses showed that energy intake was higher in sucrose consuming ad libitum-fed rats that had access to a running wheel (ACT SUC) than in those that had no running...
wheel available in their cage (AL SUC; Table 1 and Figure 3B). An ANOVA on total energy intake until day 16 indicated significant main effects of and an interaction between factors (Supplementary Table S1). Post hoc analyses revealed that energy intake in animals with limited access to food was significantly higher from day 6 onwards compared to the first day of the experiment in those rats that had access to a running wheel (FR ACT SUC; Supplementary Table S1 and Fig. S3B), whereas it increased only from day 9 onwards in sedentary rats (FR SUC; Supplementary Table S1 and Fig. S3B). Interestingly, in contrast to chow consumption, energy intake was not different between ad libitum-fed and food-restricted animals with access to a running wheel (ACT vs. FR-ACT), except for day 3 ($p < 0.05$).

### 3.4. Food restriction-induced hyperactivity-associated body and fat pad weight loss was attenuated by sucrose consumption

An ANOVA on body weight until day 6 revealed significant main effects of and interactions between factors (Table 1). Post hoc analyses indicated that among the animals with ad libitum access to food, but with no access to a running wheel, body weight was higher in those consuming saccharin (AL SUC) as compared to their counterparts drinking sucrose in addition to plain water (AL SUC) on day 6 (Table 1 and Figure 4). In addition, these analyses revealed that in sucrose-consuming rats, food-restricted animals with access to a running wheel (FR-ACT SUC) had a lower body weight on days 4–6 compared to those that had limited access to food, but no running wheel (FR SUC; Table 1 and Figure 4). Similarly, in saccharin-consuming rats, food-restricted animals with access to a running wheel (FR-ACT SUC) had a lower body weight on day 6 than those that had limited access to food, but no running wheel (FR SUC; Table 1 and Figure 4). Finally, post hoc analyses showed that among food-restricted animals with access to a running wheel body weight on day 6 was higher in those rats that consumed sucrose (FR-ACT SUC) as compared to those drinking saccharin in addition to water (FR-ACT SUC; Table 1 and Figure 4). An ANOVA on body weight of animals with access to sucrose until day 16 indicated main effects of and an interaction between factors (Supplementary Table S1). Post hoc analyses revealed that body weight among animals with access to a running wheel was lower in food-restricted compared to ad libitum-fed rats from day 6 onwards (FR-ACT SUC vs. ACT SUC; Supplementary Table S1 and Fig. S4), whereas among sedentary animals body weight was lower in food-restricted compared to ad libitum-fed rats from day 8 onwards (FR SUC vs. AL SUC; Supplementary Table S1 and Fig. S4).

To test for differences in fat pad weights relative to body weight, an ANOVA was conducted on animals killed at day 6. For the relative subcutaneous inguinal and visceral retroperitoneal and epididymal fat pads this analysis showed significant main effects of and interactions between factors (Table 2). Post hoc analyses indicated that sucrose consumption increased its relative weight in ad libitum-fed (AL SUC vs. AL SAC; Table 2) and food-restricted animals (FR SUC vs. FR SAC; $p < 0.01$), as well as in rats that had access to a running wheel while being subject to food restriction (FR-ACT SUC vs. FR ACT SAC; Table 2), but not in those rats that had free access to a running wheel and food (Table 2). To test for differences in fat pad weight relative to body weight, an ANOVA was conducted on animals with access to sucrose that were killed at day 16. This analysis showed significant main effects, but no interactions between factors (Supplementary Table S2).

### 3.5. Food restriction-induced hyperactivity-associated changes in plasma corticosterone and adrenal and thymus weight were attenuated by sucrose consumption

An ANOVA on plasma corticosterone concentrations at 1500 h on day 6 showed significant main effects of and interactions between factors (Table 2). Post hoc analyses indicated that food restriction increased plasma corticosterone in rats consuming saccharin (FR SAC) as compared to ad libitum-fed animals (AL SAC; Table 2), but not in those animals having access to sucrose in addition to plain water (FR SUC vs. AL SUC). Accordingly, corticosterone was higher in food-restricted rats with access to saccharin solution compared to those consuming sucrose (FR SAC vs. FR SAC; Table 2). Finally, access to a running wheel alone did not modify plasma corticosterone, but food restriction and running wheel access increased concentrations of this hormone in both saccharin (FR SAC vs. FR-ACT SAC; Table 2)- and sucrose (FR SAC vs. FR-ACT SAC; Table 2)-consuming animals. Among food-restricted animals with access to a running wheel plasma corticosterone was lower in rats consuming sucrose compared to those drinking saccharin solution in addition to plain water (FR-ACT SAC vs. FR-ACT SAC; Table 2). An ANOVA was conducted on plasma corticosterone of animals with access to sucrose that were killed at day 16. This analysis showed significant main effects of and an interaction between factors (Supplementary Table S1). Post hoc analyses revealed that plasma corticosterone concentrations were significantly higher in animals that were food-restricted while having access to a running wheel as compared to animals that were food restricted or that had access to a running wheel (FR-ACT SAC vs. FR SAC and FR-ACT SAC vs. ACT SAC; Supplementary Table S2) (Table 3). An ANOVA on adrenal weight relative to body weight on day 6 indicated significant main effects of and interactions between factors (Table 2). Post hoc analyses revealed that in rats consuming saccharin in addition to plain water relative adrenal weight was higher in food-restricted animals with and without access to a running wheel (FR-SAC and FR-ACT SAC) compared to free-feeding rats (AL SAC; Table 2). However, in rats consuming sucrose in addition to plain water relative adrenal weight was higher in food-restricted animals with access to a running wheel (FR-ACT SAC vs. FR SAC and FR-ACT SAC vs. ACT SAC) as compared to ad libitum fed rats regardless of whether or not they had access to a running wheel and food-restricted animals without a running wheel (AL SAC, ACT SAC and FR SAC; Table 2). Thus, among animals subject to food restriction-induced wheel running relative adrenal weight was higher in those with access to a saccharin solution than in those consuming sucrose (FR-ACT SAC vs. FR-ACT SAC; Table 2). An ANOVA conducted on adrenal weight relative to body weight of animals with access to sucrose killed at day 16 indicated significant main effects without interaction between factors (Supplementary Table S2).
Table 2  Results of statistical analyses on adipose and glucocorticoid state at day 6.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Main effects</th>
<th>Interactions</th>
<th>Post hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal fat/body weight</td>
<td>Food: $F_{1,53} = 93.7; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,53} = 9.60; p &lt; 0.01$</td>
<td>AL SUC &gt; AL SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Wheel: $F_{1,53} = 48.4; p &lt; 0.0001$</td>
<td>Food × wheel × sweet: $F_{1,53} = 4.36; p &lt; 0.05$</td>
<td>FR SUC &gt; FR SAC; $p &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,53} = 26.9; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,53} = 7.53; p &lt; 0.01$</td>
<td>FR-ACT SUC &gt; FR-ACT SAC; $p &lt; 0.05$</td>
</tr>
<tr>
<td>Retroperitoneal fat/body weight</td>
<td>Food: $F_{1,53} = 454; p &lt; 0.0001$</td>
<td>Food × sweet: $F_{1,53} = 7.53; p &lt; 0.01$</td>
<td>FR SAC &gt; FR SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Wheel: $F_{1,53} = 59.1; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,53} = 7.00; p &lt; 0.05$</td>
<td>FR &lt; FR-ACT; $p &lt; 0.01$–0.001</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,53} = 28.8; p &lt; 0.0001$</td>
<td></td>
<td>FR-ACT SUC &lt; FR-ACT SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td>Epididymal fat/body weight</td>
<td>Food: $F_{1,53} = 79.4; p &lt; 0.0001$</td>
<td>Food × sweet: $F_{1,53} = 14.8; p &lt; 0.001$</td>
<td>FR SAC &gt; ACT SAC &amp; AL SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Wheel: $F_{1,53} = 41.2; p &lt; 0.0001$</td>
<td></td>
<td>FR &lt; FR-ACT &amp; ACT SUC, FR SAC &amp; AL SAC; $p &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,53} = 20.5; p &lt; 0.0001$</td>
<td></td>
<td>FR-ACT SUC &lt; FR-ACT SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td>[Corticosterone]</td>
<td>Food: $F_{1,24} = 114; p &lt; 0.0001$</td>
<td>Food × wheel: $F_{1,24} = 11.7; p &lt; 0.01$</td>
<td>FR SAC &gt; AL SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Wheel: $F_{1,24} = 17.2; p &lt; 0.001$</td>
<td>Food × wheel × sweet: $F_{1,24} = 31.1; p &lt; 0.001$</td>
<td>FR SAC &gt; FR SUC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,24} = 28.9; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,24} = 11.6; p &lt; 0.01$</td>
<td>FR &lt; FR-ACT &amp; ACT SUC, FR SAC &amp; AL SAC; $p &lt; 0.01$</td>
</tr>
<tr>
<td>Adrenal/body weight</td>
<td>Food: $F_{1,53} = 61.4; p &lt; 0.0001$</td>
<td>Food × wheel: $F_{1,53} = 11.6; p &lt; 0.01$</td>
<td>FR SAC &amp; FR-ACT SAC &gt; AL SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Wheel: $F_{1,53} = 31.4; p &lt; 0.0001$</td>
<td>Food × wheel × sweet: $F_{1,53} = 8.29; p &lt; 0.01$</td>
<td>$p &lt; 0.01$ &amp; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,53} = 10.3; p &lt; 0.0001$</td>
<td>Wheel × sweet: $F_{1,53} = 5.70; p &lt; 0.05$</td>
<td>FR-ACT SUC &lt; ACT SUC, FR SAC &amp; AL SAC; $p &lt; 0.01$</td>
</tr>
<tr>
<td>Thymus/body weight</td>
<td>Food: $F_{1,53} = 35.3; p &lt; 0.0001$</td>
<td>Food × sweet: $F_{1,53} = 4.99; p &lt; 0.05$</td>
<td>FR SAC &gt; ACT SUC &amp; AL SAC; $p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Wheel: $F_{1,53} = 15.5; p &lt; 0.001$</td>
<td>Food × wheel × sweet: $F_{1,53} = 3.47; p &lt; 0.07$</td>
<td>FR-ACT SUC &lt; ACT SUC &amp; FR SAC; $p &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>Sweet: $F_{1,53} = 4.58; p &lt; 0.05$</td>
<td></td>
<td>FR SAC &gt; FR SAC; $p &lt; 0.001$</td>
</tr>
</tbody>
</table>

Main effects of and interactions between food availability (Food), running wheel access (Wheel) and sweetening molecule (Sweet) on fat pad, adrenal and thymus weight relative to body weight (g organ/kg body weight) and plasma corticosterone (ng/ml) at day 6 as identified by ANOVAs. Post hoc analyses indicate significant differences between groups that were relevant to our hypotheses or unexpected. AL: rats with ad libitum access to food, but not to a running wheel; ACT: animals with ad libitum access to both food and a running wheel; FR: rats subject to food restriction in the absence of a running wheel; FR-ACT: animals subject to food restriction with access to a running wheel; SAC: freely available saccharin-sweetened water in addition to plain drinking water; SUC: freely available sucrose-sweetened water in addition to plain drinking water.
Table 3  Adipose and glucocorticoid state at day 6.

<table>
<thead>
<tr>
<th>Wheel running</th>
<th>ACT SAC</th>
<th>ACT SUC</th>
<th>FR-ACT SAC</th>
<th>FR-ACT SUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal fat</td>
<td>1.887 ± 0.182</td>
<td>1.745 ± 0.195</td>
<td>0.062 ± 0.067</td>
<td>0.787 ± 0.276*</td>
</tr>
<tr>
<td>Retrop. fat</td>
<td>1.540 ± 0.122</td>
<td>1.593 ± 0.093</td>
<td>0.000 ± 0.000</td>
<td>0.349 ± 0.121</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>2.243 ± 0.238</td>
<td>2.562 ± 0.187</td>
<td>0.207 ± 0.100</td>
<td>1.549 ± 0.341</td>
</tr>
<tr>
<td>[Corticosterone]</td>
<td>59.53 ± 8.44</td>
<td>70.40 ± 15.50</td>
<td>256.00 ± 16.55</td>
<td>150.42 ± 13.82***</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.242 ± 0.007</td>
<td>0.211 ± 0.014</td>
<td>0.395 ± 0.013</td>
<td>0.303 ± 0.028***</td>
</tr>
<tr>
<td>Thymus</td>
<td>2.575 ± 0.053</td>
<td>2.853 ± 0.084</td>
<td>1.318 ± 0.082</td>
<td>1.718 ± 0.180</td>
</tr>
</tbody>
</table>

Sedentary

<table>
<thead>
<tr>
<th>Wheel running</th>
<th>AL SAC</th>
<th>AL SUC</th>
<th>FR SAC</th>
<th>FR SUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal fat</td>
<td>2.094 ± 0.123</td>
<td>3.399 ± 0.308</td>
<td>0.933 ± 0.165</td>
<td>1.940 ± 0.208</td>
</tr>
<tr>
<td>Retrop. fat</td>
<td>2.150 ± 0.074</td>
<td>2.485 ± 0.083</td>
<td>0.134 ± 0.067</td>
<td>0.984 ± 0.168</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>3.297 ± 0.123</td>
<td>3.188 ± 0.209</td>
<td>1.396 ± 0.270</td>
<td>2.638 ± 0.146</td>
</tr>
<tr>
<td>[Corticosterone]</td>
<td>61.30 ± 8.18</td>
<td>54.40 ± 10.42</td>
<td>183.47 ± 20.51</td>
<td>74.32 ± 12.90</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.191 ± 0.006</td>
<td>0.214 ± 0.011</td>
<td>0.272 ± 0.018</td>
<td>0.230 ± 0.014</td>
</tr>
<tr>
<td>Thymus</td>
<td>3.293 ± 0.433</td>
<td>2.984 ± 0.179</td>
<td>1.867 ± 0.140</td>
<td>2.903 ± 0.338</td>
</tr>
</tbody>
</table>

Measures (mean ± SEM) of relative fat pad, adrenal and thymus weight (g organ/kg body weight) and plasma corticosterone (ng/ml) obtained at day 6 in rats with ad libitum access to food, but not to a running wheel (AL), with ad libitum access to both food and a running wheel (ACT), subject to food restriction in the absence of a running wheel (FR) and subject food restriction with access to a running wheel (FR-ACT). All animals had ad libitum access to either saccharin (SAC)- or sucrose (SUC)-sweetened water in addition to plain drinking water. *p < 0.05, ***p < 0.001 between FR-ACT SAC and FR-ACT SUC in post hoc tests.

An ANOVA on thymus weight on day 6 relative to body weight showed significant main effects without interaction between factors as well as a significant and a tendency for an interaction (Table 2). Post hoc analyses indicated that food restriction was sufficient to decrease relative thymus weight in animals consuming saccharin (FR SAC vs. AL SAC and FR SAC vs. ACT SAC; Table 2), whereas food restriction needed to be associated to running wheel access to result in lower thymus weight in rats drinking sucrose in addition to plain water (FR-ACT SAC vs. ACT SAC and FR-ACT SUC vs. FR SAC; Table 2). Thus, among animals subject to food restriction without access to a running wheel relative thymus weight was lower in those with access to a saccharin solution than in those consuming sucrose (FR SAC vs. FR SAC; Table 2). An ANOVA was conducted on thymus weight relative to body weight of animals with access to sucrose that were killed at day 16 and revealed significant main effects and an interaction between factors (Supplementary Table S2). Post hoc analyses indicated that relative thymus weight was significantly lower in animals that were food-restricted while having access to a running wheel as compared to animals that were food restricted or that had access to a running wheel (FR-ACT SAC vs. FR SUC and FR-ACT SUC vs. ACT SAC; Supplementary Table S2).

3.6. Food restriction-induced hyperactivity-associated c-Fos expression in the PVH, ARH and nucleus accumbens was attenuated by sucrose consumption

An ANOVA on c-Fos expression in the PVH of animals with access to a running wheel showed a significant effect of food availability ($F_{1,19} = 12.4; p < 0.01$) and a significant interaction between food availability and sweetening molecule ($F_{1,17} = 8.8; p < 0.01$). Post hoc analysis indicated that c-Fos expression in the PVH was significantly higher in food-restricted animals with access to a running wheel and saccharin as compared to their ad libitum-fed counterparts (FR-ACT SAC vs. ACT SAC; $p < 0.01$) and compared to food-restricted rats with access to a running wheel and that consumed sucrose (FR-ACT SAC vs. FR-ACT SUC; $p < 0.01$; Figure 5A).

An ANOVA on c-Fos expression in the ARH of animals with access to a running wheel revealed a significant effect of food availability ($F_{1,19} = 11.0; p < 0.01$) and tendencies for an effect of sweetening agent ($F_{1,9} = 3.21; p = 0.09$) and an interaction between food availability and sweetening molecule ($F_{1,19} = 3.67; p = 0.07$). Post hoc analysis indicated that c-Fos expression in the ARH was significantly higher in food-restricted rats with access to a running wheel and saccharin as compared to their ad libitum-fed counterparts (FR-ACT SAC vs. ACT SAC; $p < 0.01$) and compared to food-restricted animals with access to a running wheel and that drank sucrose (FR-ACT SAC vs. FR-ACT SUC; $p < 0.05$; Figure 5B).

An ANOVA on c-Fos expression in the dorsal nucleus accumbens of animals with access to a running wheel showed a significant effect of food availability ($F_{1,19} = 20.6; p < 0.001$) and sweetening molecule ($F_{1,19} = 6.91; p < 0.05$) as well as a significant interaction between these factors ($F_{1,19} = 6.19; p < 0.05$). Post hoc analysis indicated that c-Fos expression in the dorsal nucleus accumbens was significantly higher in food-restricted animals with access to a running wheel and saccharin as compared to their ad libitum-fed counterparts (FR-ACT SAC vs. ACT SAC; $p < 0.01$) and compared to food-restricted rats with access to a running wheel and that consumed sucrose (FR-ACT SAC vs. FR-ACT SUC; $p < 0.001$; Figure 5C).

An ANOVA on FosB expression in the nucleus accumbens of animals with access to a running wheel did not indicate any significant differences between groups (food restriction vs. ad libitum-fed or sucrose vs. saccharin drinking solution in addition to plain water; data not shown).

4. Discussion

The present work showed that free access to sucrose, but not to saccharin, during limited food access attenuated rises in plasma corticosterone, running wheel activity, weight loss
Sucrose attenuates food restriction-induced hyperactivity and forebrain activation

Figure 5  c-Fos expression in the paraventricular (A) and arcuate hypothalamus (B) and in the nucleus accumbens (C) of Lewis rats with ad libitum access to both food and a running wheel (ACT) and subject to food restriction with access to a running wheel (FR-ACT). All animals had ad libitum access to either saccharin-(SAC) or sucrose-(SUC)-sweetened water in addition to plain drinking water. Graphs show the mean number of c-Fos-positive nuclei per section while photomicrographs illustrate labeling in a representative brain section of an animal of each group. 3v: third ventricle; lv: lateral ventricle. Scale bar represents 200 μm in A and C and 100 μm in B. *p < 0.05; **p < 0.01; ***p < 0.001 between FR-ACT SAC and FR-ACT SUC.

and c-Fos expression in the PVH, ARH and nucleus accumbens 4–6 days later.

Our findings extend previous studies showing that free consumption of glucose early on during food restriction attenuates wheel running (Takeda et al., 2003) and that the reinforcement value of sucrose substitutes to a certain extent that of wheel running in food-restricted rats (Belke et al., 2006). In contrast, when sucrose and saccharin solutions are provided one week after food restriction has started, they have no effect on daily wheel running and weight loss (Brown et al., 2008). Although published findings thus indicate that sweetness is an early signal attenuating food restriction-induced hyperactivity, they do not allow to distinguish between the reward-addiction and the fleeing-famine hypotheses, since glucose and sucrose are rewarding and addictive (Avena et al., 2008) as well as rich in easy-to-assimilate energy. According to the reward-addiction hypothesis, food restriction along with increased physical activity activates brain reward circuits and may become addictive (Bergh and Sodersten, 1996; Fladung et al., 2010). If food restriction along with wheel running is rewarding and addictive, then the known high reward and addictiveness of sweet taste of both saccharin and sucrose (Lenoir et al., 2007; Avena et al., 2008) should attenuate food restriction-induced hyperactivity and weight loss. The fleeing-famine hypothesis postulates that refusal of known scarce energy-low or hard-to-assimilate food sources and hyperactivity facilitate migration towards new habitats potentially containing new energy-rich or easily-assimilable foodstuffs (Guisinger, 2003). Therefore, if scarcity of energy-low food sources provides the trigger for hyperactivity then the presence of energy-rich sucrose, yielding fructose and
glucose, and not that of saccharin should attenuate food restriction-induced wheel running and weight loss. Our observations that (1) animals with access to saccharin showed similar wheel running and weight loss as previously reported in rats that were not exposed to sweet solutions (Girard et al., 2001; Duclos et al., 2005, 2009), (2) sucrose consumption attenuated food restriction-induced running wheel activity and weight loss compared to saccharin intake and (3) food-restricted animals with access to a running wheel eventually increased sucrose, but not saccharin, consumption thus corroborate the fleeing-famine hypothesis.

Sedentary rats with free access to food rapidly drank substantial, yet similar, amounts of sucrose or saccharin solutions and preferred these to plain water. In contrast, food-restricted rats immediately consumed more sucrose than saccharin suggesting that metabolic signals, rather than gustatory properties, increase the salience for energy-rich solutions in these animals. Compared to sedentary rats that were restricted to the same amount of food, food-restricted animals with access to a running wheel did not increase sucrose consumption during the first days. This may be explained by a competition between the reinforcement values of wheel running and sucrose consumption (Belke et al., 2006) or by wheel-running-induced conditioned taste aversion (Lett and Grant, 1996; Lett et al., 1998; Forrissil et al., 2007). However, after a couple of days, food-restricted animals with access to a running wheel increased sucrose, but not saccharin, consumption. Thus, regardless of the mechanisms underlying the low consumption of sweet solutions during the first days, our present findings rather suggest that the energy-richness and easy availability of sucrose eventually interrupted a fleeing-famine-like hyperactivity response.

Takeda et al. (2003) mention that, in parallel to attenuating food restriction-induced wheel running, glucose consumption prevented hypoglycemia and reduced plasma corticosterone concentrations, even though this latter claim was not supported by statistical analysis. Corticosterone production is stimulated by hypoglycemia and its circulating concentrations are increased during chronic food restriction (Garcia-Belenguer et al., 1993). Corticosterone stimulates hepatic gluconeogenesis (Holmes et al., 2001) and the intake of carbohydrates (Tempel and Leibowitz, 1994) and sweet solutions (Bell et al., 2000; Bhatnagar et al., 2000). Moreover, we have shown that corticosterone mediates food restriction-induced wheel running as this response is virtually absent in adrenalectomized Lewis rats and restored by acute or chronic corticosterone replacement (Duclos et al., 2009). Our present observations show that among food-restricted animals with a running wheel, preprandial plasma corticosterone 6 days after the start of the experiment was lower in rats consuming sucrose than in those having access to a saccharin solution. The attenuating effect of sucrose consumption also concerned changes in adrenalin and thymus weight that are generally considered as indicators of peripheral glucocorticoid state (Dallman et al., 1999). Sucrose yields fructose and glucose and consumption of the former avoids hypoglycemia in starved or food-restricted rats (Ruhe et al., 1996; Levine and Saltzman, 2000) while ingestion of the latter prevents hypoglycemia during food restriction-induced wheel running (Badman and Flier, 2005). Taken together, it is likely that sucrose consumption in the present study reduced restriction-induced hypoglycemia and, by consequence, circulating corticosterone and wheel running.

In order to identify neurocircuits that are activated during the paradoxical association of food restriction and excessive wheel running, we used detection of Fos transcription factors. c-Fos is often used as a neuronal activation marker in response to acute stimuli, but has also proven useful in chronic conditions characterized by reduced food intake and weight loss, such as cancer (Konsman and Blomqvist, 2005). The pattern of c-Fos expression observed in wheel-running food-restricted animals with access to saccharin, in addition to water, was reminiscent of that observed in previous studies on animals with no access to sweet solutions. Indeed, acute running, whether or not it occurs in anticipation of food, can enhance c-Fos expression in the PVH, ARH and nucleus accumbens (Liste et al., 1997; Vargas-Perez et al., 2003; Soya et al., 2007; Verhagen et al., 2011).

Our observation that c-Fos protein expression in the PVH of food-restricted wheel running rats with access to sucrose was lower than in those with access to saccharin extents a recent finding showing that allowing sucrose intake along with a scheduled meal attenuates PVH c-fos mRNA expression at the moment of the expected meal (Mitra et al., 2011). The PVH receives important input from brainstem neuropeptide Y neurons in the nucleus of the solitary tract (NTS) (Fuzesi et al., 2007). The NTS, in turn, receives projections from glucose-sensitive vagal fibers that converge onto intrinsic glucose-sensitive neurons (Adachi et al., 1984; Grabauskas et al., 2010). Interestingly, sucrose intake is more efficient to induce c-fos expression in the NTS than an expected regular meal (Emond and Weingarten, 1995; Mitra et al., 2011) and daily intake of sucrose-enriched food increases brainstem neuropeptide Y expression compared to scheduled standard chow (Olszewski et al., 2009). Furthermore, chronic intracerebroventricular infusion of neuropeptide Y results in reduced corticotropin-releasing hormone mRNA expression in the PVH (Fuzesi et al., 2007). A majority of corticotropin-releasing hormone-containing PVH neurons release this hormone at the median eminence to stimulate pituitary adrenocorticotropic hormone secretion, which, in turn, acts on the adrenal to produce corticosterone. Finally, PVH lesions (Mistlberger and Rusak, 1988), like adrenalecctomy (Duclos et al., 2009), lower the probability of increased locomotor activity prior to a scheduled meal. In view of the available literature, the presently observed attenuation of PVH c-Fos expression by sucrose consumption in food-restricted rats with access to a running wheel may involve caudal brainstem circuits and explain, at least in part, lower circulating corticosterone concentrations and reduced wheel-running in these animals.

The ARH plays an important role in the regulation of food intake and energy expenditure (Badman and Flier, 2005). We found that among food-restricted animals with access to a running wheel, ARH c-Fos expression was higher in rats consuming saccharin than in those drinking sucrose and compared to ad libitum-fed rats. In the ARH proopiomelanocortin-containing neurons bear corticosterone receptors (Cintra and Bortolotti, 1992) while neuropeptide Y-positive neurons express glucose sensors (Fioramonti et al., 2007). The attenuating effect of sucrose intake on ARH c-Fos expression in food-restricted animals with access to a running wheel may therefore occur as a consequence of reduced...
hypoglycemia or lower plasma corticosterone. Glucocorticoid replacement or dexamethasone administration increases ARH proopiomelanocortin expression in adrenalectomized animals (Tong et al., 1990). Interestingly, proopiomelanocortin gene transfer to hypothalamus and brainstem increases physical activity (Zhang et al., 2011). Given the catabolic effects of proopiomelanocortin-derived alpha melanocyte-stimulating hormone, it is possible that corticosterone through its action on ARH neurons stimulates wheel running and mobilizes the necessary energy reserves.

Motivation for running in rodents selected for wheel running correlates with c-Fos-immunoreactivity in the dorsal nucleus accumbens (Rhodes et al., 2003). We observed that among rats with access to a running wheel, c-Fos expression in the dorsal nucleus accumbens was higher in food-restricted than in freely-feeding animals. In addition, we found that sucrose consumption reduced accumbens c-Fos expression in food-restricted animals with access to a running wheel. This observation may seem in contradiction with a published study showing that sucrose intake along with a scheduled meal increases nucleus accumbens c-fos mRNA expression prior to the meal (Mitra et al., 2011). Unlike the present work, the latter study assessed locomotor activity in a different set of animals housed in metabolic cages that did not contain running wheels and did not observe increased c-fos expression in the nucleus accumbens of food-restricted animals compared to free-feeding rats. The use of running devices by rodents increases nucleus accumbens c-Fos expression (Liste et al., 1997; Vargas-Perez et al., 2003). Differences in assessment of food restriction-induced hyperactivity between studies may therefore underlie discrepancies in nucleus accumbens c-fos expression. Since lesions of the nucleus accumbens affect preprandial locomotor activity (Mendoza et al., 2005), we propose that lower nucleus accumbens activity in food-restricted rats with access to a running wheel and sucrose plays a role in reduced wheel activity displayed by these animals. This effect may involve reduced corticosterone concentrations as this hormone stimulates nucleus accumbens dopamine release and locomotor activity (Piazza et al., 1996).

In addition to c-Fos, wheel running can increase FosB expression in the nucleus accumbens of ad libitum-fed rodents (Werme et al., 2002). The absence of increased FosB expression in the nucleus accumbens of wheel running Lewis rats in the present study may therefore come as a surprise. One needs to bear in mind, however, that the truncated form of FosB accumulates with time and that FosB was assessed 6 days after the start of the experiment in the present study, whereas it was detected after one month of wheel running in previous work. It is, therefore, likely that FosB was not yet detectable in the present work.

In conclusion, we have shown that access to a sucrose solution in wheel-running food-restricted rats attenuated rises in plasma corticosterone, wheel running activity, weight loss as well as c-Fos expression in the PVH, ARH and nucleus accumbens and resulted in increased intake of this solution after a couple of days as compared to animals having access to an equally-sweet saccharin solution in the same experimental conditions. We propose that sucrose consumption by wheel-running food-restricted rats attenuates activation of the PVH through caudal brainstem circuits and thus lowers plasma corticosterone. Since corticosterone mediates food restriction-induced wheel running (Duclos et al., 2009), its lower concentrations can explain reduced wheel running and weight loss observed in these animals.

Increased physical activity is present in 30–80% of anorexia nervosa patients (Davis et al., 1994; Klein et al., 2007) and associated with increased plasma cortisol (Klein et al., 2007). Although an oral or intravenous glucose load, sufficient to suppress cortisol levels in healthy volunteers, is without effect in anorexia nervosa patients (Tamai et al., 1991; Misra et al., 2004), the effect of repeated glucose or sucrose regimens is unknown. It is interesting to note in this respect that anorexia nervosa patients dislike high-fat foods (Drewnowsk et al., 1988), but do not seem to consider sucrose aversive when they are satiated (Garfinkel et al., 1979). In view of our present findings along with available clinical data, it would be of particular interest to study the effect of prolonged glucose or sucrose administration to anorexia nervosa patients showing high levels of physical activity.

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Conflict of interest

No financial or other interest exists with regard to the submitted manuscript that might be construed as a conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2012.09.012.

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