Tonic and phasic effects of corticosterone on food restriction-induced hyperactivity in rats

Martine Duclos 1, Clémence Gatti, Baptiste Bessière, Pierre Mormède*

PsyNuGen, INRA, UMR 1286, Université Bordeaux 2, F-33076 Bordeaux, France

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1. Introduction

An elevated physical activity is present in 31—80% of patients suffering from anorexia nervosa. Excessive exercise has been viewed as a strategy of anorectic patients to lose weight. However, subjective reports of patients’ experiences reveal a compulsive component, indicating that exercise might not be under cognitive control of the patient (Holtkamp et al., 2003). These two important clinical features seen in most patients with anorexia nervosa, i.e. low food intake and excessive physical activity, are present in the experimental animal model referred to as semi-starvation-induced hyperactivity (Pirke et al., 1993; Exner et al., 2000) or activity-based anorexia (Epling and Pierce, 1988; Burden et al., 1993). The principle of this animal paradigm is as follows: when rats have access to a running wheel with food freely...
available, limited activity normally occurs but when the animals are restricted to a fixed amount of food, presented once per day, consistent running occurs which becomes excessive and associated rapidly with dramatically reduced food intake. If this vicious circle is not disrupted by the investigator, rats starve and run themselves to death (Epling and Pierce, 1988). This decoupling between food intake and energy expenditure is associated with activation of the hypothalamo–pituitary–adrenal (HPA) axis (Burden et al., 1993; Duclos et al., 1999), but the pathophysiological role of the HPA axis in the increased physical activity associated with food restriction remains unidentified (Pirke et al., 1993).

Recent insights on the importance of glucocorticoids in feeding and energy balance emphasized the role of the HPA axis in energy homeostasis (Akan et al., 1994). Glucocorticoids also have complex effects in the central nervous system to increase the salience of activities associated with food seeking such as wheel running (Leshner, 1971). Indeed, Challet et al. (1995) have demonstrated the involvement of cort in the rise in locomotor activity characteristic of the end of the fasting period in Sprague–Dawley rats submitted to fasting and wheel activity, since the increase in locomotor activity was suppressed by adrenalectomy and restored by cort replacement. In brain, glucocorticoids may exert positive hedonic effects via an increase of dopamine release in the nucleus accumbens (Piazza et al., 1996) thereby increasing the behavioral effects of psychostimulants (e.g. Rivet et al., 1989; Cador et al., 1993) or increasing the compulsive nature of some activities as clearly demonstrated for drug-taking behaviors (Piazza and Le Moal, 1997). The pleasurable component of increased physical activity in both human anorexia nervosa and animal models of semi-starvation-induced hyperactivity/activity-based anorexia may reinforce this behavior, and inhibit food intake via reward mechanisms (Bergh and Sodersten, 1996; Exner et al., 2000).

The comparison of three strains of rats emphasized the co-occurrence of higher HPA axis activation and higher wheel running in food-restricted rats (Lewis > Brown Norway > Fischer 344 rats for both HPA axis activation and wheel activity), supporting the hypothesis of a link between higher HPA axis activation and higher running associated with food restriction (Duclos et al., 2005). An important issue that remains to be solved is the direction of this link between the activation of the HPA axis and the increased wheel activity of food-restricted rats. On one hand, negative energy balance and physical activity by themselves activate the HPA axis (Pirke et al., 1993; Dalman et al., 1999). On the other hand, chronic cort administration has been shown to increase locomotor activity (Wolkowitz, 1994) and cort is necessary for the occurrence of schedule-induced wheel running after adrenalectomy (Challet et al., 1995).

In an attempt to characterize the influence of cort on wheel activity in food-restricted rats, we used adrenalectomized (ADX) rats replaced with pellets containing increasing amounts of cort that caused different steady-state plasma concentrations from low to high (stress-induced) HPA activity in rats fed ad libitum (AL, experiment 1) or food-restricted (FR, experiment 2). Finally, we and others reported previously that in rats a prefeeding cort peak developed under restricted feeding followed, after food intake, by a dramatic decrease in plasma cort such that cort was normalized to control values observed at the same time as in ad libitum fed rats (e.g. Honma et al., 1984; Garcia-Belenguer et al., 1993; Duclos et al., 2005). In parallel with the prefeeding cort peak, wheel activity was increased in the period preceding the anticipated meal whereas in the postprandial period, this activity was totally suppressed (Duclos et al., 2005). In the third experiment we investigated the acute effect of cort injection mimicking the prefeeding cort peak on prefeeding wheel activity on ADX rats replaced with a variety of pellets. The objective was to determine if the increase in wheel activity before food intake was influenced by this plasma cort release in anticipation of a meal.

2. Materials and methods

2.1. Subjects

Three separate studies were carried out. Experiments were conducted on male Lewis rats (IFFA CREDO, Les Oncins, France). Although anorexia nervosa mainly affects women and there is a sexually dimorphic sensitivity to semi-starvation-induced hyperactivity (SIH) in rats, male rats being more resistant to (Doerries et al., 1991), we chose to study only male rats to discard any confusing effect of variations of estrogen and progesterin secretion during the estrus cycle. The animals were 4-week-old upon arrival at our laboratory, and were housed 4/cage (with food and water ad libitum) in an animal quarter at constant temperature (23–25 °C) and a 12-h/12-h light–dark cycle (lights on at 07:00 h). They were left undisturbed for 2 weeks before the beginning of the experiments. This study was conducted in conformity with the French recommendations on animal experimentation. The rats were given unrestricted access to water throughout the experiment and fed on standard laboratory chow. When their body weight reached 170–180 g (6–7-week-old), rats were randomly assigned to experimental groups and either bilaterally adrenalectomized or Sham adrenalectomized under intraperitoneal pentobarbital anaesthesia. In ADX rats, SC pellets of 100 mg of wax (control) or a fused mixture of cort (12.5 mg, 50 mg or 100 mg) and cholesterol were inserted at the time of surgery ~2 cm rostral to the skin incision in the nape of the neck. Consequently, there were five experimental groups: Sham-ADX with wax pellet and ADX with wax pellet or 12.5 mg, 50 mg, 100 mg cort pellets, named respectively, Sham, ADX-0, ADX-12.5, ADX-50 and ADX-100. After surgery all rats were provided with both water and 0.9% saline solution to drink. Body weight and food intake were measured daily in all rats.

2.2. Experiment 1: effects of chronic corticosterone levels on wheel activity in ad libitum fed rats

On day 0 of the experiment (D0), 7 days after adrenal surgery and pellet insertion, at 1500 h, animals were placed in individual cages where they had ad libitum access to food and a permanent access to a running wheel (circumference: 94.2 cm) (n = 4–6 rats/group). The rats were kept in a room that housed all cages, wheels and recording equipment. Access to this room was limited to feeding and weighting times to prevent interference with activity. Wheel turns were
automatically monitored by computer and stored at 60-s intervals in Microsoft Excel files for each wheel from 15:00 h on D0 to 15:00 h on (Dx + 1). Throughout the experiment, rats were weighed at 15:00 h.

2.3. Experiment 2: effects of chronic corticosterone levels on wheel activity in food-restricted rats

On day 0, 7 days after adrenal surgery and pellet insertion, at 15:00 h, another set of rats were placed in individual cages where they had a daily wheel access for 22.5 h while food deprived. For the remaining 1.5 h (15:00 h–16:30 h) daily each the animals were locked out of the wheels and given free access to food (n = 6–8 rats/group).

2.4. Experiment 3: effects of acute increase of corticosterone levels on wheel activity in ad libitum and food-restricted rats

This experiment was undertaken to simulate in adrenalectomized animals the prefeeding increase in cort levels superimposed to various basal cort levels maintained by cort pellets. One set of rats was submitted to the same protocol as described in experiment 2 (food restriction) and injected SC once daily at 11:00 h with either vehicle (INJ\textsubscript{VEH}) or cort (INJ\textsubscript{CORT}) (5 mg/kg body weight in 0.2 ml) on D2–D4 (n = 6–8 rats/group). A second set of rats was also subjected to the same protocol as described in experiment 1 (ad libitum food intake) and SC injected with vehicle or cort on D2–D4 (n = 4–6 rats/group). All rats were killed on D6. The dosage of cort was chosen to obtain plasma levels similar to those measured in Lewis rats submitted to restraint stress (Martin et al., 2000) or food restriction (Duclos et al., 2005). Corticosterone (Sigma–Aldrich, Germany) was dissolved in saline–100% ethanol (90:10) to obtain a 5-mg/ml stock solution (Wang and Johnson, 1990). Control rats received vehicle only.

2.5. Kinetics of plasma corticosterone levels after corticosterone injection

This experiment was undertaken before experiment 3 to determine the time when the injection of cort should be realized to simulate the prefeeding peak of cort in food-restricted rats. Twenty rats of experiment 3 were used, 3 days after adrenal surgery and pellet insertion (and 4 days before the beginning of the experiment). They were placed in individual cages with ad libitum access to food and drinks (water and salted water). At 11:00 h, rats were quickly removed from their home cage, gently wrapped in a towel in an adjacent room, and blood (30 μl) was collected from a small nick made on the tail with a scalpel (no. 15 blade). The entire sampling procedure was accomplished within 2 min of touching the cage to ensure low basal levels of cort for Sham rats. Rats (n = 4/group) were then injected SC (0.2 ml) with cort (see experiment 3) and blood samples were taken 1 h, 2 h, 4 h and 6 h later. Tail blood (30 μl) was collected in 1.5 ml polyethylene tubes containing 4 μl of 10% EDTA, and stored in ice until centrifugation. Plasma samples were stored at −80°C until analysis.

2.6. Post-mortem analyses

Based on our previous study where 6–8 days were necessary for food-restricted wheel-running rats to lose 25% of their initial body weight (Duclos et al., 2005), it was planned that rats would be killed on D7. However, in experiment 2, ADX-0 and ADX-12.5 food-restricted rats were removed from the wheel and killed earlier than D7 because of exhaustion of the rats (analysis of body weight data showed a total depletion at the time of sacrifice). For these reasons, the other experimental groups (Sham, ADX-50 and ADX-100) of experiment 2 were killed on D6 instead of D7 as well as all five experimental groups of experiment 3. Between 15:00 h and 15:20 h, the animals were removed from their cage and killed by decapitation in an adjacent room within 30 s of removal from their home cage.

Immediately after decapitation, trunk blood was collected in 10 ml polyethylene tubes containing 0.1 ml of 10% EDTA, and stored in ice until centrifugation. Plasma samples were stored at −80°C until analysis. The thymus gland was removed and weighed as an indicator of peripheral glucocorticoid status (Cador et al., 1993). White adipose tissue pads (retroperitoneal, epididymal and SC [inguinal]) were dissected and weighed.

2.7. Corticosterone measurements

Plasma cort was measured after alcohol extraction by a competitive protein-binding assay (Chaouloff et al., 1995) using rhesus monkey serum as the source of transcortin, [3H]cort as the tracer, and dextran-coated charcoal to absorb the unbound fraction (sensitivity 4 ng/ml, specificity >95%, and interassay coefficient of variation 8.0%). Samples were analyzed in duplicate.

2.8. Statistical analyses

Data are presented as means ± S.E.M. All data were analyzed by one-, two- or three-way analysis of variance (ANOVA), corrected when appropriate for repeated measures. In case of a significant main effect or interaction, a posteriori comparisons were performed using the Tukey HSD test. Statistical significance was accepted at p < 0.05.

3. Results

3.1. Experiment 1: effects of chronic corticosterone levels on in ad libitum fed rats

All rats began the experiments at the same weight (201.1 ± 3.6 g). Cumulative weight variations for the five groups of rats during the experiment are shown in Fig. 1. The statistical analysis on the last day data (Δweight) showed a lower weight gain in Sham vs. ADX-50 groups (21.1 ± 1.9 g vs. 36.7 ± 2.5 g, Sham vs. ADX-50, p = 0.02), whereas there was no difference with the three other groups. The relative adipose tissue weight did not show significant differences across groups (Table 1). The differences in body weight gain were not related to differences in food intake (data not shown) but were associated with differences in wheel activity (Fig. 2, top
A one-way ANOVA with repeated measures revealed no effect of treatment ($F_{4,20} = 1.4, p = 0.25$) but a significant effect of day ($F_{6,120} = 9.6, p < 10^{-6}$) and a significant interaction between day and treatment ($F_{24,120} = 1.9, p = 0.009$). Post hoc tests showed that wheel activity in ad libitum food intake condition was low and similar across the four groups of ADX rats whatever their cort replacement level with no significant effect of day ($\sim 1500$ turns $\sim 1.4$ km/22.5 h). By contrast, wheel activity progressively increased from day to day in Sham rats as they ran more on D6 and D7 than on D1–D3.

In Table 2 the total 22.5 h of running was divided into four time periods based upon behavior of food-restricted rats of experiment 2 and previously published data (Duclos et al., 2005): (i) the night period (19:00–07:00 h), (ii) the first 5 h of the light period (07:00–12:00 h), (iii) 12:00–15:00 h (the 3 h immediately before the next feed (prefeeding period) for food-restricted rats of experiment 2) and (iv) 16:30–19:00 h (the first 2.5 h following feeding (postprandial period) for food-restricted rats of experiments 2 and 3). The daily pattern of wheel activity was roughly similar between the five groups of rats. Detailed examination of 22.5 h wheel turns (realized on D4 as corresponding to the last day of the experiment for the majority of the rats) showed that the main activity occurred during the night period for all groups of rats (78% and 72% of the 22.5 h activity for Sham and all ADX-CORT groups, respectively) (Table 2). In the five groups of rats, the remaining activity occurred mainly during the first 5 h of the light period (07:00–12:00 h) (17% and 23% of

![Figure 1](image1.png) Cumulative weight variations in each experimental group (means ± S.E.M.). ADX, adrenalectomy; the figure following ADX is the corticosterone content (mg) of the pellet implanted in ADX rats at the time of surgery; AL, ad libitum (experiment 1); FR, food-restricted (experiment 2).

![Figure 2](image2.png) Changes in pattern of wheel activity in ad libitum fed rats of experiment 1 (top) and in food-restricted rats of experiment 2 (bottom). Each data point indicates the mean number of wheel turns during each 22.5 h period (16:30–15:00 h). *Sham group: $p < 0.05$ vs. D1–D3. *ADX-12.5, ADX-50, ADX-100 and Sham groups: $p < 0.05$ vs. D1 and D2. &ADX-100 and Sham groups: $p < 0.05$ vs. D1 and D2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Relative adipose tissue weight (mg/g body weight). The adipose tissue weight is the sum of three white adipose tissue pads: retroperitoneal, epididymal and subcutaneous.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>Experiment 1 (ad libitum)</td>
</tr>
<tr>
<td>ADX-0</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>ADX-12.5</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>ADX-50</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>ADX-100</td>
<td>6.6 ± 1.2</td>
</tr>
<tr>
<td>Sham</td>
<td>6.3 ± 0.5</td>
</tr>
</tbody>
</table>

Within the same experiment, $p > 0.05$ between groups.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Circadian pattern of 22.5 h wheel activity (in % of total wheel activity) in experiment 1 (rats fed ad libitum) on D4. The total 22.5 h of running was divided into four time periods based upon behavior of food-restricted rats of experiment 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>Night (19:00–07:00 h)</td>
</tr>
<tr>
<td>ADX-0</td>
<td>72.2 ± 4.9</td>
</tr>
<tr>
<td>ADX-12.5</td>
<td>67.8 ± 5.3</td>
</tr>
<tr>
<td>ADX-50</td>
<td>71.5 ± 19.4</td>
</tr>
<tr>
<td>ADX-100</td>
<td>78.2 ± 9.4</td>
</tr>
<tr>
<td>Sham</td>
<td>78.3 ± 7.1</td>
</tr>
</tbody>
</table>

In Table 2 the total 22.5 h of running was divided into four time periods based upon the behavior of food-restricted rats of experiment 2 and previously published data (Duclos et al., 2005): (i) the night period (19:00–07:00 h), (ii) the first 5 h of the light period (07:00–12:00 h), (iii) 12:00–15:00 h (the 3 h immediately before the next feed (prefeeding period) for food-restricted rats of experiment 2) and (iv) 16:30–19:00 h (the first 2.5 h following feeding (postprandial period) for food-restricted rats of experiments 2 and 3). The daily pattern of wheel activity was roughly similar between the five groups of rats. Detailed examination of 22.5 h wheel turns (realized on D4 as corresponding to the last day of the experiment for the majority of the rats) showed that the main activity occurred during the night period for all groups of rats (78% and 72% of the 22.5 h activity for Sham and all ADX-CORT groups, respectively) (Table 2). In the five groups of rats, the remaining activity occurred mainly during the first 5 h of the light period (07:00–12:00 h) (17% and 23% of...
the 22.5 h activity for Sham and all ADX-CORT groups, respectively) with no difference between groups. Within each experimental group the repartition of wheel activity (in % of the 22.5 h activity) did not differ across days (data not shown).

3.2. Experiment 2: effects of chronic corticosterone levels in food-restricted rats

All rats entered the experiment at the same weight (192.3 ± 1.8 g). Fig. 1 shows that food restriction associated with free access to activity wheel decreased body weight at the same rate in all groups of rats. However, ADX-0 and ADX-12.5 rats were removed from the wheel and killed earlier than D7 (4.5 ± 0.3 days after the beginning of the experiment (D0) [range: D4–D7] for ADX-0 and 5.2 ± 0.2 days [range: D5–D7] for ADX 12.5) because of exhaustion of the rats (analysis of fat depots showed a total depletion in these rats at the time of sacrifice (Table 1)). ADX-50, ADX-100 and Sham groups were removed at the same time (D6) because maximal wheel activity occurred on D5 and D6 (see below) for ADX-50 and ADX-100 rats. No difference in food intake was observed among the groups (mean food intake for the five experimental groups on D5: 4.8 ± 0.3 g in experiment 2 vs. 19.3 ± 0.4 g in experiment 1).

By contrast, significant differences in wheel activity were found (Fig. 2, bottom panel). A one-way ANOVA with repeated measures (until D5 for all groups) revealed a effect of treatment (F4,32 = 8.1, p < 10−4), of day (F4,122 = 59.5, p < 10−4) with a significant interaction between treatment and day (F16,122 = 6.9, p < 10−4). Post hoc tests showed that exposure to food restriction progressively increased 22.5 h total running in all groups, except in ADX-0 rats, and that this increase in wheel activity occurred at a higher rate in rats with higher cort replacement level. As indicated by the total 22.5 h wheel turns for D1–D6 (but without statistical analysis for D6) shown in Fig. 2, ADX-50 ran more than ADX-0 on D3–D5 and than ADX-12.5 on D5 and ADX-100 ran more than ADX-0 on D2–D5 and more than ADX-12.5 on D4 and D5. Finally, Sham rats ran more than ADX-0 on D3–D5 and than ADX-12.5 on D5. A day effect was also found with ADX-12.5 rats increasing their wheel activity until a maximum on D4 (2364 ± 630 turns/22.5 h ∼2.2 km run), ADX-50 until D6 (5216 ± 386 turns/22.5 h ∼4.9 km run), ADX-100 until D4 (4443 ± 453 turns/22.5 h ∼4.2 km run) and Sham until D6 (7081 ± 942 turns/22.5 h ∼6.7 km run).

Detailed examination of the distribution of wheel turns over 22.5 h (realized on D4 as corresponding to the last day of the experiment for the majority of the rats) showed that most activity occurred during the night period (65% and 63% of the 22.5 h activity for Sham and the four ADX-CORT groups, respectively) (Table 3). In the four ADX-CORT groups, the remaining activity was realized during the first 5 h of the light period (07:00–12:00 h) (10.1% and 12.5% of the 22.5 h activity for Sham and the four ADX-CORT groups, respectively) and after feeding (16:30–19:00 h) (2.8% and 16.5% of the 22.5 h activity for Sham and the four ADX-CORT groups, respectively) with no difference between the five groups. A significant difference between Sham rats and all ADX groups (22.6% vs. 7.3% of the 22.5 h activity, respectively, p < 0.05) emerged in the prefeeding period corresponding to the food-anticipatory period of 3 h prior to feeding where Sham rats only increased significantly their wheel activity.

3.3. Thymus weight and plasma corticosterone

For thymus weight and plasma cort level analyses, rats of experiments 1 and 2 were pooled as in ADX rats cort levels were dependent on the cort content of the pellet implanted and not on feeding conditions (ad libitum vs. food-restricted rats). Indeed, a one-way ANOVA showed an experimental group effect ($F_{5,57}$ = 24.49, p < 10−4) on thymus weight (Fig. 3). Post hoc tests showed a difference between the Sham groups with a decrease in thymus weight with food-restriction (Sham AL (experiment 1) vs. Sham FR (experiment 2): p < 10−4). Increased cort replacement levels from ADX-0 to ADX-100 rats were associated with decreased thymus weight (significant difference between each ADX-CORT

![Table 3](https://example.com/table3.png)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Night 19:00–07:00 h</th>
<th>First light period 07:00–12:00 h</th>
<th>Prefeeding period 12:00–15:00 h</th>
<th>Postprandial period 16:30–19:00 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADX-0</td>
<td>65.1 ± 9.3</td>
<td>8.5 ± 2.9</td>
<td>5.9 ± 3.7</td>
<td>21.2 ± 11.3</td>
</tr>
<tr>
<td>ADX-12.5</td>
<td>70.2 ± 2.9</td>
<td>9.4 ± 2.1</td>
<td>8.6 ± 2.7</td>
<td>11.5 ± 3.8</td>
</tr>
<tr>
<td>ADX-50</td>
<td>57.7 ± 3.3</td>
<td>14.8 ± 3.0</td>
<td>6.3 ± 2.2</td>
<td>21.2 ± 3.7</td>
</tr>
<tr>
<td>ADX-100</td>
<td>61.7 ± 3.5</td>
<td>17.4 ± 3.3</td>
<td>8.6 ± 2.1</td>
<td>12.0 ± 2.8</td>
</tr>
<tr>
<td>Sham</td>
<td>65.1 ± 7.3</td>
<td>10.1 ± 3.4</td>
<td>22.6 ± 2.3</td>
<td>2.8 ± 3.4</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to the other experimental groups within the same period.

![Figure 3](https://example.com/f3.png)

Figure 3 Relative thymus weight for each experimental group of experiments 1 and 2. Bars represent means ± S.E.M. Bars not sharing the same letter are significantly different (p < 0.05).
group). Moreover, thymus weight of ADX-12.5 rats was not different from thymus weight of Sham AL rats and thymus weight of ADX-100 rats was not different from thymus weight of Sham FR rats.

Plasma cort levels in samples collected from the six experimental groups of rats at 15:00 h by decapitation on the day of sacrifice are shown in Table 4. A one-way ANOVA showed an experimental group effect (\(F_{5,57} = 13.2, p < 10^{-4}\)), post hoc tests showed a difference between the Sham and ADX groups with a stimulatory effect of food restriction on cort levels (day of sacrifice: AL vs. FR: \(p < 0.01\)). For ADX rats, plasma cort concentrations were roughly proportional to pellet composition.

### 3.4. Experiment 3: effects of acute increase of corticosterone levels in ad libitum and food-restricted rats

#### 3.4.1. Kinetics of plasma corticosterone after corticosterone injection

A one-way ANOVA with repeated measures revealed a significant group effect (\(F_{4,15} = 2.9, p = 0.05\)), time effect (\(F_{4,60} = 626.4, p < 10^{-4}\)) and interaction between group and time (\(F_{16,60} = 4.3, p < 10^{-3}\)) on cort levels. Fig. 4 shows that post-injection basal cort values differed significantly between ADX-100 and the other experimental groups. One hour, 2 h and 4 h after cort injection, plasma cort was significantly increased compared to post-injection levels with no difference between groups. Six hours after injection plasma cort levels were not significantly different from basal cort values but differed between ADX-100 and the other experimental groups.

#### 3.4.2. Effects of acute corticosterone surge on wheel activity

A chronic treatment (ADX-CORT pellet) \(\times\) acute treatment (cort vs. vehicle) \(\times\) day ANOVA revealed that in rats with ad libitum food access, and compared to vehicle rats, cort injection on D2–D4 induced no effect on post-injection wheel activity (from 11:00 h to 17:00 h) on the corresponding day whatever the experimental group considered and therefore the basal cort value (Fig. 5, upper panel, shows data on D5; similar results were obtained on D2–D4 but are not shown).

In rats with food restriction, the same analysis revealed that there was an overall stimulatory effect of cort injection on post-injection wheel activity \((F_{1,53} = 4.7, p = 0.01)\) with a chronic treatment effect \((F_{4,53} = 3.7, p = 0.01)\) and a trend to an interaction between chronic and acute treatment conditions \((F_{4,53} = 2.4, p = 0.05)\). Post hoc tests in ADX-0 rats revealed a marked increase in wheel activity on D2–D4 in rats injected with cort compared to vehicle-injected rats. In ADX-12.5 and ADX-50 rats, cort injection induced a marked increase in wheel activity on D3 and D4 compared to vehicle injection. In ADX-100 rats, cort induced a marked increase in wheel activity on D2–D4 compared to vehicle. In Sham rats no effect of cort injection on wheel activity compared to vehicle was found, probably because the post-injection

Table 4  Plasma corticosterone (ng/ml) in experiments 1–3.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
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<tr>
<td></td>
<td>AL</td>
<td>FR</td>
<td>AL</td>
</tr>
<tr>
<td>ADX-0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ADX-12.5</td>
<td>45.7 ± 7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.5 ± 8.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.8 ± 6.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADX-50</td>
<td>91.3 ± 4.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>102.2 ± 12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.4 ± 17.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADX-100</td>
<td>148.2 ± 15.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>179.2 ± 19.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.2 ± 10.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sham</td>
<td>41.3 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159.2 ± 28.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.1 ± 11.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. For corticosterone analysis, rats belonging to the same experimental group of experiments 1 and 2 were pooled, except for Sham rats (Sham AL of experiment 1 and Sham FR of experiment 2). For corticosterone analysis of rats of experiment 3, rats belonging to the same experimental group were also pooled, except for Sham rats, with a separate analysis for vehicle and corticosterone injected rats. ND, not detectable.

Experiments 1 and 2: \(p < 0.05\) compared to Sham AL; \(p < 0.05\) compared to Sham FR.

Experiment 3: \(p < 0.05\) compared to Sham AL; \(p < 0.05\) compared to Sham FR.

<sup>a</sup> Acute treatment.
chronic treatment (C2)

The results are not different from those obtained in experiments 1 and 2 (data not shown).

3.4.4. Plasma corticosterone

Figure 5 Effects of SC injection of corticosterone or vehicle at 11:00 h on D4 on wheel activity of rats fed ad libitum (top) and food-restricted (bottom). The effects on D2 and D3 are similar to those of intact rats. Differences in duration of the experimental period (7 days (present study) vs 14 days (Challet et al., 1995) who reported that ADX reduced activity fed rats. Altogether, these results demonstrate the critical role of cort in the development of food restriction-induced hyperactivity, an experimental model of anorexia nervosa.

4. Discussion

Our results demonstrate that corticosterone plays both a tonic and phasic effect on food restriction-induced hyperactivity. First, the development of wheel activity with food restriction was completely blocked by adrenalectomy and dose-dependently restored by basal cort levels maintained by cort pellets in ADX rats. Second, the preprandial surge of hyperactivity was also blocked by adrenalectomy and was not restored by increasing steady-state hormone levels by cort pellets but only by a phasic increase of cort levels induced by hormone injection before the feeding period. Finally, an interaction was demonstrated between these phasic and tonic effects since the efficiency of acute injection was dependent on steady-state levels of cort. No such effects were found in ad libitum fed rats. Altogether, these results demonstrate the critical role of cort in the development of food restriction-induced hyperactivity, an experimental model of anorexia nervosa.

4.1. Methodological considerations

In the present experiment, rats were adrenalectomized and implanted with SC pellets with various concentrations of cort, the rat natural glucocorticoid hormone. The advantage of the pellet method includes the establishment of constant cort concentrations with no evidence of circadian variation (Meyer et al., 1979). Data of plasma cort concentrations, body weight gain, and thymus weight (an indicator of peripheral glucocorticoid state) show that the levels of cort achieved by the 12.5 mg cort pellets were in the low physiological range (corresponding to cort values obtained during the light phase in intact sedentary and wheel-running rats) whereas the cort levels obtained after the implantation of the 50 mg cort pellets were in the high physiological range (corresponding to cort values obtained during the dark phase in intact sedentary and wheel-running rats (Gomez et al., 1996; Duclos et al., 2005). It is important to note that the plasma cort values of the ADX-100 mg CORT pellets remained at the levels of plasma cort observed in chronically stressed rats (Duclos et al., 2001) or food-restricted rats (Garcia-Belenguer et al., 1993), suggesting that the range of cort concentrations achieved in the present experiments represents reference values from low to high (stress-induced) HPA axis activity.

4.2. Effects of chronic corticosterone levels on wheel activity in ad libitum fed rats

The objective of experiment 1 was to determine the impact of different steady-state cort levels on spontaneous wheel activity in ad libitum fed rats. Our results show that in ADX rats increasing concentrations of steady-state cort have no significant effect on 22.5 h wheel activity and the activity of these groups does not differ from that of Sham rats (~2 km/22.5 h). These results are in agreement with those of Micco et al. (1980) but contrast with those of Leshner (1971) and Challet et al. (1995) who reported that ADX reduced activity levels and replacement therapy with cort returned levels to those of intact rats. Differences in duration of the experiments (7 days (present study) vs. 14 days (Challet et al.,

Figure 5 Effects of SC injection of corticosterone or vehicle at 11:00 h on D4 on wheel activity of rats fed ad libitum (top) and food-restricted (bottom). The effects on D2 and D3 are similar to those of intact rats. Differences in duration of the experimental period (7 days (present study) vs 14 days (Challet et al., 1995) who reported that ADX reduced activity levels and replacement therapy with cort returned levels to those of intact rats. Differences in duration of the experiments (7 days (present study) vs. 14 days (Challet et al.,

3.4.3. Thymus weight

A three-way ANOVA (chronic treatment × acute treatment × feeding condition [AL vs. FR]) showed significant chronic treatment (F1,93 = 74.8, p < 10^-6), acute treatment (F1,93 = 17.1, p < 10^-4), and feeding condition (F1,93 = 20.4, p < 10^-5) effects. Post hoc tests comparing thymus weight of rats injected with vehicle or rats injected with cort revealed a significant decrease in thymus weight after 3 days of cort injection in ADX-0 and ADX-12.5 (p = 0.02 for each group) compared to their vehicle counterparts. The other results are the same than in experiments 1 and 2 (data not shown).

3.4.4. Plasma corticosterone

The results are not different from those obtained in experiments 1 and 2 (Table 4). As in experiment 1, for ADX rats, plasma cort concentrations were roughly proportional to pellet composition. Moreover, when Sham AL and FR rats were compared, a stimulatory effect of food restriction on cort levels was observed in the FR groups (AL vs. FR: p < 0.01). At the time of the death, i.e. 2 days after the last cort injection, there was no difference between plasma cort of vehicle rats and rats injected with cort.
The light phase and high during the dark phase (Table 2). ad libitum intact study and the two previous ones. the experiments) may explain the differences between our study and the two previous ones. In agreement with Eikleboom and Mills (1988), wheel running shows a pronounced entrained circadian rhythm in intact ad libitum fed rats: running levels are very low during the light phase and high during the dark phase (Table 2). Surprisingly, when the circadian pattern of activity in ADX rats is examined, no difference is found between rats with constant 24 h plasma cort concentration—which does not mimic the nychthemeral rhythm of plasma cort concentration (ADX-CORT rats)—and Sham rats, suggesting that the circadian pattern of wheel activity of ad libitum fed rats is unrelated to the nychthemeral fluctuations of cort levels, as previously shown (Iuvone and Van Hartesveldt, 1977; Micco et al., 1980; Challet et al., 1995). Taken together, these results suggest that cort is not a major regulator of the diurnal periodicity of activity in ad libitum fed rats. Nevertheless, locomotor activity only represents a part of a set of variables which display a circadian rhythm and which could be affected by corticosterone depletion, on the one hand, and the absence of circadian rhythm of corticosterone, on the other hand. Recent studies involve corticosterone in the synchronisation of both peripheral and central clock genes affecting metabolism and behavior (Balsalobre et al., 2000; Segall et al., 2006).

4.3. Effects of chronic corticosterone levels on wheel activity in food-restricted rats

The objective of this experiment was to determine the impact of different steady-state cort levels on spontaneous wheel activity in food-restricted rats. Our results show that adrenalectomy suppresses the development of activity and that, in ADX rats, increasing steady-state concentrations of cort are associated with a dose-dependent increase of 22.5 h wheel activity and a faster increase in activity across successive days.

The comparison of results of experiments 1 and 2 clearly shows that it is only in condition of food restriction and in presence of cort that rats develop excessive running. The large increase in the spontaneous wheel activity of Sham rats during food restriction is consistent with many previous studies (Sclafani and Rendel, 1978; Koubi et al., 1991; Challet et al., 1995; Duclos et al., 2005). This rise in wheel activity is suppressed by adrenalectomy and restored when rats were provided with cort replacement with a dose—response effect, demonstrating that cort plays a critical role in this food restriction-induced rise in wheel running. It is worth noting that cort pellets (12.5—50 mg) restoring physiological levels of hormone also restore activity levels similar to that of Sham rats. In a previous study we have also shown by comparing rat strains with different HPA axis activity that the locomotor response to food restriction was proportional to cort secretion (Duclos et al., 2005).

Severe food restriction increases HPA axis activity in humans (Boyar et al., 1977; Duclos et al., 1999) and rats with an increase in adrenal weight and mean cort levels, and a decrease in thymus weight (Garcia-Belenguer et al., 1993; Duclos et al., 2005; present study). On the other hand, cort is necessary for the rise in locomotor activity during prolonged fasting and occurrence of schedule-induced wheel running (Leshner, 1971; Challet et al., 1995). Taken together, the present and past studies fit with the hypothesis of a central role of cort in the pathophysiology of food restriction-induced paradoxical wheel activity in rats, along the cycle "food restriction—cort secretion—increased activity".

The analysis of the circadian pattern of locomotor activity adds another evidence of the critical involvement of cort in food restriction-induced wheel activity. Although the general profile of activity is not much influenced by food restriction, a marked difference in 22.5 h wheel activity emerged between Sham ad libitum and food-restricted rats on one hand, and between Sham food-restricted rats and all the other ADX-CORT food-restricted groups on the other hand, with the development of a burst of wheel activity during the 3 h prior to feeding, corresponding to the food-anticipatory period in the Sham food-restricted group only (23% of their 22.5 h activity vs. 3% in Sham ad libitum rats and 6—9% in the four food-restricted ADX-CORT groups). These data confirm and complete the demonstration of an increased locomotor activity prior to mealtime in food-restricted rats (Boulos et al., 1980; Duclos et al., 2005). This finding is quite striking given that in parallel with the prefeeding wheel activity, cort peaked in the period preceding the anticipated meal (Boulos et al., 1980; Duclos et al., 2005) whereas after food intake (postprandial period), wheel activity is totally suppressed and cort returns to control values at the same time in both ad libitum sedentary and ad libitum wheel-running rats (Duclos et al., 2005). It suggests that the prefeeding peak of cort secretion could also mediate the concomitant increase in wheel activity. This hypothesis was directly addressed in experiment 3.

4.4. Effects of acute corticosterone injection on the prefeeding peak of wheel activity

Rats were injected SC at 11:00 h with a significant increase in cort levels from 12:00 h to 15:00 h mimicking the prefeeding cort increase. The dosage of cort used in this study was chosen because it resulted in an elevation of cort levels identical to that obtained in Lewis rats submitted to restraint stress (Martin et al., 2000) or food restriction (Duclos et al., 2005).

In food-restricted ADX rats, cort injection acutely increases wheel activity. By contrast, in rats with ad libitum food access, cort injection induced no acute effect on wheel activity whatever the experimental group and therefore the basal cort level. This acute effect of cort is observed even in rats with no prior cort impregnation (ADX-0) but is larger with increasing mean 22.5 h cort levels, demonstrating a potentiation of basal and acute effects of cort on wheel activity. Another way to test the dependence of food-restricted hyperactivity on cort would be to show that pre-treatment with type II glucocorticoid receptor antagonist, RU38486, could prevent hyperactivity in intact (non-ADX) food-restricted rats (Jacobson and Sapolsky, 1993). Altogether, these results demonstrate that cort also plays an acute role in the paradoxical prefeeding peak in wheel activity observed in
food-restricted rats (increased wheel activity during the 3 h following cort injection) and that the timing of events is as follows: increased cort levels inducing a peak in wheel activity. It is worth mentioning that acute increases in corticosterone have also been shown to normalize ACTH responses to stress (Jacobson and Sapolsky, 1993).

Food-anticipatory activity that rats express before a daily timed meal is considered as the behavioral output of a feeding-entrainable oscillator whose functional neuroanatomy is still unknown (Mistlberger and Mumby, 1992). During food-anticipatory activity, induction of c-Fos expression have been reported in widespread neuronal network within the central nervous system (de Vasconcelos et al., 2006) and it has been hypothesized that this induction of c-Fos expression could be triggered by endocrine signals such as glucocorticoids (Piazza et al., 1996). In accordance with this hypothesis, Pecoraro et al. (2005), in ADX rats bearing varying doses of corticosterone replacement pellets and submitted to a successive incentive contrast procedure conducted on a plus maze, reported increased anticipatory activity in food-restricted rats, this effect being entirely corticosterone dependent. However, the authors stated that: “although we did observe some motivational effects of corticosterone in ad libitum fed animals, we have no strong arguments for or against the requirement of a concurrent state of deprivation for the induction of these effects.” Our results reproduce and extend these results suggesting strongly that the brain network within which corticosterone acts must be stimulated to reveal the behavioral effects of corticosterone.

This prefeeding increase in cort levels is in agreement with the metabolic roles of cort (Akana et al., 1992; Dallman et al., 1999). Indeed, cort plays an important role in the mobilization of energy reserves during energy deficit from fasting or energy restriction or during prolonged exercise by stimulating lipolysis and increasing protein catabolism. La Fleur et al. (2004) proposed that the role of cort on feeding may be to increase the level of drive as they reported that cort functions in a dose-related manner to increase caloric intake. This interpretation of the role of cort on caloric intake is consistent with other motivational effects of cort such as the relationship demonstrated between the amount of drug self-administration and circulating cort, and may be mediated by its interaction with dopaminergic systems (Piazza and Le Moal, 1997; Pecoraro et al., 2005).

5. Conclusion

The present experiments bring several important findings. First, wheel running induced by food restriction is nearly non-existent in adrenalectomized food-restricted rats and increases in a dose-related manner with cort replacement. By contrast, cort does not influence wheel running in ad libitum fed rats. Second, cort exerts both tonic and phasic stimulatory effects on wheel activity in food-restricted rats. The phasic stimulation of activity is expressed during the preprandial peak of cort, suppressed by adrenalectomy (with and without chronic cort replacement) and experimentally restored by acute cort injection, whatever the basal cort levels, i.e. in the presence or absence of cort.

Although a number of hypothetical relationships have been evoked between wheel running and food restriction, our data emphasize the critical role of cort. We propose that cort may be the link between low fat mass (peripheral energetic) and increased physical activity, favoring fueling through lipolysis and proteolysis, increasing the drive for food and reinforcing self-starvation via reward mechanisms, establishing a deleterious vicious cycle.

As previously demonstrated for rat strains genetically differing in their HPA axis reactivity where large strain- and HPA axis reactivity-dependent differences in activity responses to food restriction were found, humans with genetically higher HPA axis reactivity to stress may represent subjects particularly prone to develop a syndrome of anorexia-hyperactivity in conditions of voluntary food restriction. The present results have special relevance for the pathophysiology of anorexia nervosa as well as other compulsive behaviors.

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Conflict of interest

None declared.

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