Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 81 (2005) 41 – 46

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

The effects of chronic food restriction on hypothalamic–pituitary–adrenal activity depend on morning versus evening availability of food

Xavier Belda, Sheila Ons, Javier Carrasco, Antonio Armario*

Institut Neurociències, Facultat de Ciències (Departament de Biologia Cellular, de Fisiologia i d'Immunologia), Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain
Unitat de Fisiologia Animal, Facultat de Ciències (Departament de Biologia Cellular, de Fisiologia i d'Immunologia), Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain

Received 9 August 2004; received in revised form 31 December 2004; accepted 10 February 2005
Available online 22 April 2005

Abstract

Partial food restriction (FR) protocols have been used not only to study behavioral and physiological consequences of decrease food intake, but as a necessary treatment of the animals in some operant learning tasks. It is well-established in rodents that restricting food availability to a few hours in the morning causes an alteration of the daily rhythm of corticosterone, thus making it difficult to evaluate whether or not such treatments are stressful. In the present experiment adult male Sprague–Dawley rats were subjected to two different FR schedules: food availability after 1100 h (LFR) or after 1900 h (DFR). After 14 days, animals from both groups, together with corresponding controls, were killed under resting conditions, either in the morning or in the evening, just before daily access to food in FR rats. Both FR schedules reduced body weight gain to the same extent, but their impact on the hypothalamic–pituitary–adrenal (HPA) axis was different: DFR increased relative, but not absolute, adrenal weight and morning and evening levels of corticosterone, whereas LFR increased both absolute and relative adrenal weights and increased morning corticosterone levels to a greater extent than DFR rats. Neither serum ACTH nor corticotropin-releasing factor (CRF) mRNA levels in the paraventricular nucleus of the hypothalamus were altered by DFR or LFR protocols, suggesting that factors other than CRF and ACTH are involved in the control of adrenocortical secretion under FR. It appears that LFR caused more alterations in the HPA axis than DFR and, therefore, the latter FR schedule should be used in those protocols necessarily involving partial FR.

Keywords: Food restriction; Stress; Daily rhythm; CRF; ACTH; Corticosterone; Adrenal

1. Introduction

Chronic partial food restriction (FR) protocols are extensively used in a wide range of instrumental learning paradigms using food as a reinforcer, and also in some experiments dealing with operant drug self-administration, either because the rats are firstly trained to obtained food or because FR is considered as a stressor and the interaction between stress and drug administration is the purpose of the experiments (i.e. Goeders and Guerin, 1994; Kantak et al., 2002; Merlo-Pich et al., 1997). Whereas the use of such paradigms is, in some cases, necessary, attention should be paid to the degree of FR and the time of day at which food is available. Regarding the latter point, it is known that some of the physiological consequences of FR appear to be critically dependent on the time of day at which the animals have access to food and the degree of FR. Thus, daily restriction of food availability to 1–2 h in the morning induces in the rat the appearance of a pre-feeding peak of plasma corticosterone (Krieger, 1974; Moberg et al., 1975). On the contrary, this pre-feeding peak of corticosterone does not appears when food is available for a much longer period of time (i.e. Honma et al., 1983). Chronic FR protocols not

* Corresponding author. Unitat de Fisiologia Animal, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. Tel.: +34 935811664; fax: +34 935812390.
E-mail address: antonio.armario@uab.es (A. Armario).

0091-3057/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.pbb.2005.02.009

only induce a pre-feeding peak of corticosterone, but they can also modify the normal daily peak of corticosterone at lights off. It appears that restricting food availability to a short period of time not close to lights off did not alter, or only reduced, the normal daily peak of corticosterone at that time (i.e. Armario et al., 1987; Moberg et al., 1975; Morimoto et al., 1977), whereas reducing the interval between food availability and lights off times drastically impaired the normal corticosterone peak at lights off (Morimoto et al., 1977; Xu et al., 1999).

The earlier results of increased morning levels of corticosterone after chronic FR were erroneously interpreted as an evidence that such treatments were stressful. However, a certain degree of FR can induce an overall hyperactivity of the HPA axis reminiscent of chronic stressful situations. Thus, Garcia-Belenguer et al. (1993), using a FR schedule with food availability just after lights off, to avoid any drastic alteration of the daily corticosterone rhythm, observed that FR to 85% of that of control rats did not cause any important modification of the daily corticosterone rhythm, but FR to 50% markedly increased corticosterone levels at lights on. These data indicate that FR above a certain threshold may be stressful for the rats. What is still unclear is whether the overall impact of FR may be different depending on the time of food availability. Thus, in the present experiment we compared the effects of imposing a FR to 60% of that of controls, either in the morning (as usual in most laboratories) or in the evening, which is clearly more appropriate for the integrity of daily rhythms. We chose such a level of FR because it is the degree of reduction of food intake caused by daily exposure to a severe stressor such as immobilization (Marti et al., 1994). Such level of FR has been demonstrated in our lab to fully explain the changes caused by chronic immobilization stress on luteinizing hormone secretion (Marti et al., 1993), suggesting that at least some of the consequences of stress may be explained by secondary changes in FR rather than by the impact of the stressor itself.

2. Materials and methods

2.1. Animals and experimental groups

Forty-eight male Sprague–Dawley rats from the breeding centre of the Universitat Autònoma de Barcelona, approximately 4 months old at the beginning of the experiment, were used. They were housed, two per cage, in standard conditions of temperature (22±1 °C), and a 12:12-h light–dark schedule (lights on at 0700 h). Food and water were provided ad libitum before the experimental period. The experimental protocol was approved by the committee of Ethics of the Universitat Autònoma de Barcelona and was carried out in accordance with the European Communities Council Directive (86/609/EEC).

The animals were randomly assigned to three experimental groups: (a) controls, left undisturbed throughout the experimental period; (b) FR (60% of food intake of control rats), with food availability during the morning, after 1100 h (LFR); (c) FR (60% of food intake of control rats), with food availability during the evening, after 1900 h. After 14 days, rats from the three groups were killed either in the morning (between 1030 and 1050 h) or in the evening (between 1830 and 1850 h). The two rats from a cage were killed simultaneously by decapitation under undisturbed conditions. Trunk blood was collected and maintained in ice-cold water until centrifugation and the brains were immediately taken, dipped into a recipient with isopentane (Fluka, Spain) maintained in liquid nitrogen and then stored at −80 °C until 20 μm coronal sections were obtained with a cryostat. Sections were mounted in slices and stored at −20 °C.

2.2. Radioimmunoassays

Serum ACTH and corticosterone levels were measured by radioimmunoassay (RIA). Serum corticosterone was determined by a previously described RIA (Armario and Castellanos, 1984), with two modifications: the antiserum used was obtained in sheep against corticosterone-3-CMO-urease (Chemicon Int., Temecula, CA, USA) and corticosterone-binding globulin was denaturated by trypsin method (Roche Diagnostics, Barcelona, Spain). Serum ACTH was measured by a double-antibody RIA using 125I-ACTH (Amersham, Spain) as the tracer, rat synthetic ACTH 1–39 (Sigma, Spain) as the standard and an antibody raised against rat ACTH (rb 7) kindly provided by Dr. W.C. Engeland (Department of Surgery, University of Minnesota, Minneapolis, USA). Before ACTH RIA, serum samples were extracted by solid-phase extraction with Sep-Pak cartridges C18 (Waters; Milford, Massachusetts). Cartridges were conditioned with acetonitrile 60% in trifluoroacetic acid 0.1% (buffer B). Conditioning was followed by an equilibration step using trifluoroacetic acid 0.1% (buffer A). Samples were mixed 1:1 with buffer A and loaded onto the cartridges (1 ml of mixed sample; flow rate 1 ml/min). The cartridges were extensively washed with buffer A to remove interferences in the sample matrix. Finally, 3 ml of buffer B was applied to elute ACTH. The eluents were dried by a nitrogen stream and the wet material reconstituted with 0.5 ml of assay buffer (Egg albumin 0.25%; Triton X-100 0.1%; EDTA 25 mM; sodium phosphate 50 mM; pH 7.4). All samples to be compared were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was 8% for corticosterone and 5% for ACTH.

2.3. In situ hybridisation

The CRF probe was generated from *Eco*RI fragment of rat *CRF* cDNA, subcloned into pGEM-4Z (Promega, USA) and linearised with *Hin*dIII. The plasmid was generously

provided by Dr. S. Rivest (Laval University, Quebec, Canada). Radioactive antisense cRNA copies were generated using a transcription kit (Promega, USA). Once digested, linearised plasmid was incubated in a transcription buffer (40 mM Tris–Cl, pH 7.9, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl), 10 mM DTT, 0.2 mM GTP/ATP/CTP, 200μCi [α-^{35}S] UTP (specific activity >1000 Ci/mmol, Amersham Pharmacia Biotech, UK), 40U RNasin and 20U of SP6 RNA polymerase for 60 min at 37 °C. The DNA template was digested with RNase-free DNase (Promega, USA; 1 U DNase in 0.25 μg/μl tRNA and 9.4 mM Tris/9.4 mM $MgCl_2$) and extracted with phenol–chloroform–isoamylalcohol 25:24:1 (Sigma, Spain). The cRNA was precipitated with the ammonium acetate method, resuspended in 10 mM Tris/1 mM EDTA, pH 8.0, and stored at −20 °C.

The protocol used for in situ hybridisation hystochemistry was adapted from Simmons et al. (1989). All the solutions were pre-treated with diethylpyrocarbonate (DEPC) and sterilized before use. Sections were post-fixed in 4% PFA+Borax rinsed in KPBS, digested with proteinase K (Roche, Switzerland; 0.01 mg/ml in 100 mM Tris–HCl, pH 8.0, and 50 mM EDTA, pH 8), rinsed in DEPC-treated water and 0.1 M triethanolamine, pH 8.0 (TEA, Sigma, Spain) and acetylated in 0.25% acetic anhydre in 0.01 M TEA. Finally, they were washed in 2× saline–sodium citrate (SSC), dehydrated through a graded concentration of ethanol and then air-dried. Thereafter, 90 μl of hybridisation buffer (50% formamide, NaCl 0.3 M, Tris–Cl 10 mM, pH 8.0, EDTA 1 mM, pH 8.0, 1× Denhardts, 10% dextrane sulphate, yeast tRNA 500 μg/ml and 10 mM dithiothreitol (DTT) containing the labelled probe (1×10^6 cpm/90 μl) were spotted onto each slide and sealed with a coverslip. Sections were incubated for 16–18 h in a humid chamber at 60 °C. After hybridisation, the slides were washed in 4 X SSC containing 1 mM DTT (Sigma, Spain), digested with RNase A (Amersham Pharmacia Biotech, UK; 0.02 mg/ml in 0.5 M NaCl, 10 mM Tris–HCl, pH 8.0, and 1 mM EDTA, pH 8.0), washed in descending concentrations of SSC containing 1 mM DTT, dehydrated through a series of ethanol solutions (50, 70, 95 and 2 × 100%) and air-dried. The slides were then exposed to a XAR-5 Kodak Biomax MR autoradiography film (Kodak, Spain) for 24–72 h, depending on the intensity of the signal in each zone.

2.4. Image analysis

Densitometrics analyses were done on the films. The mRNA levels were semiquantitatively determined in two to three sections per brain area and animal. Sections to be analyzed were digitalized and quantified using Image software (W. Rasband, NIH, USA) by setting up the best threshold to avoid detecting only background signal and obtaining measures in arbitrary units (pixel area×average sum gray) All samples to be statistically compared were processed in the same assay to avoid inter-assay variability.

2.5. Statistical analysis

The statistical significance of the FR schedules on body weight gain and adrenal weight were analyzed by one-way ANOVA, followed by post hoc comparisons with the Student–Newman–Keuls (SNK) test ($p<0.05$). The statistical significance of serum ACTH, serum corticosterone and CRF mRNA levels were analyzed by two-way ANOVA, with FR schedules and time of day as the main factors. When the interaction FR schedule by time was significant, a breakdown of the interaction was done. With all variables, data were log transformed to achieve homogeneity of variances, if appropriate.

3. Results

The one-way ANOVA revealed significant effects of FR schedules on body weight gain ($F(2,42)=20.6$, $p<0.001$). Post hoc comparisons revealed that both LFR and DFR groups showed a lower rate of body weight gain as compared to controls, being the lost of weight similar in the two FR groups (Table 1). Both absolute and relative adrenal weights (Table 1) were significantly influenced by FR ($F(2,40)=8.2$, $p<0.001$ and $F(2,40)=23.1$, $p<0.001$, respectively). However, time of FR was important in that absolute adrenal weight increased in LFR as compared to both DFR and controls, and relative adrenal weight increased in DFR group as compared to controls and even more in LFR, which differed from both DFR and control groups.

The two-way ANOVA revealed no effects of either FR of time of day on CRF mRNA levels in the PVN and serum ACTH levels (Fig. 1). In contrast, the two-way ANOVA of serum corticosterone levels revealed significant effects of FR ($F(2,39)=7.0$, $p<0.003$), time of day ($F(1,39)=111.9$, $p<0.001$) and the interaction FR by time of day ($F(2,39)=17.5$, $p<0.001$). Further appropriate comparisons revealed (Fig. 2) that serum corticosterone differed among groups at both times ($p<0.001$). In the morning, both DFR rats and LFR rats showed higher corticosterone levels than controls ($p<0.012$ and $p<0.001$, respectively), but LFR showed higher levels than DFR ($p<0.003$). In the evening, DFR rats showed higher corticosterone levels than both

Table 1
Effects of food restriction on body and adrenal weight

Group	Initial body weight (BW; g)	Body weight gain (g)	Adrenal weight (mg)	Relative adrenal weight (mg/100 g BW)
Control	510±7[a]	35.8±2.9[a]	57.3±1.3[a]	10.6±0.3[a]
LFR	494±14[a]	−34.0±1.9[b]	65.2±1.6[b]	14.3±0.4[b]
DFR	502±8[a]	−29.7±1.5[b]	57.9±1.7[a]	12.3±0.4[c]

Means±S.E.M. ($n=13–16$) are represented. Groups labelled with different letters are significantly different (SNK test). LFR: light food restriction; DFR: dark food restriction.

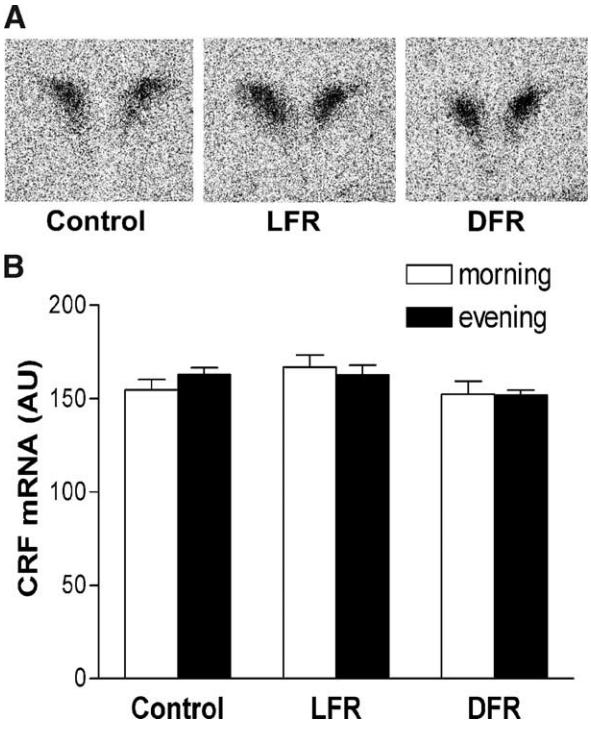

Fig. 1. Effect of two different chronic schedules of food restriction (FR), with morning (1100 h) or evening (1900 h) availability of food (LFR and DFR, respectively) on CRF mRNA levels in the paraventricular nucleus of the hypothalamus (PVN). Panel A shows the representative autorradiographies of the PVN, and panel B, the means and S.E.M. ($n=6-8$ per group) corresponding to the different treatments. Open bars indicate morning and closed bars evening levels. No significant differences of either FR schedule or time of day were observed.

control and LFR animals ($p<0.02$ and $p<0.001$, respectively). Whereas higher evening versus morning corticosterone levels were observed in both controls ($p<0.001$) and DFR rats ($p<0.001$), such pattern was not found in LFR animals.

4. Discussion

The present data indicate that the time of day at which food is available in a FR schedule, while not modifying the degree of weight loss, has an important influence on some components of the HPA axis of adult male rats, namely adrenal weight and basal corticosterone levels.

The rats were subjected to FR for 2 weeks by making available to the rats only a 60% of the food consumed by ad libitum fed rats (controls). Since rats eat preferentially in the dark period of the daily cycle (i.e. Morimoto et al., 1977), time of day at which food is available may profoundly modify the normal daily pattern of food intake and other associated rhythms, including that of activity and body temperature (Krieger, 1974; Krieger et al., 1977; Stephan, 1984). Therefore, food was made available at 1100 h (LFR group) or at 1900 h (DFR group). In the latter case, the daily pattern of food intake may be roughly similar to that of controls, whereas in the former case, changes in the daily pattern of food intake are profoundly disturbed and so other food associated daily rhythms. The time of food availability did not modify the degree of weight loss, suggesting that caloric value of food is not affected by the time of food processing in the gastrointestinal tract.

Control rats showed the well-described daily pattern of circulating corticosterone, with very low levels in the morning (average 0.6 μg/dl) and a peak in the evening (average 13.9 μg/dl). Morning levels of corticosterone were higher in DFR than control rats and LFR increased even more basal corticosterone levels. In the evening, corticosterone levels were similar in LFR and controls and elevated in DFR rats. The pre-feeding or pre-watering corticosterone peak in LFR rats is a very consistent phenomenon in the literature (i.e. Krieger, 1974; Krieger et al., 1977; Moberg et al., 1975; Morimoto et al., 1977), and normal levels of corticosterone at lights off has been usually reported with food access during 1–2 h in the morning (i.e. Armario et al.,

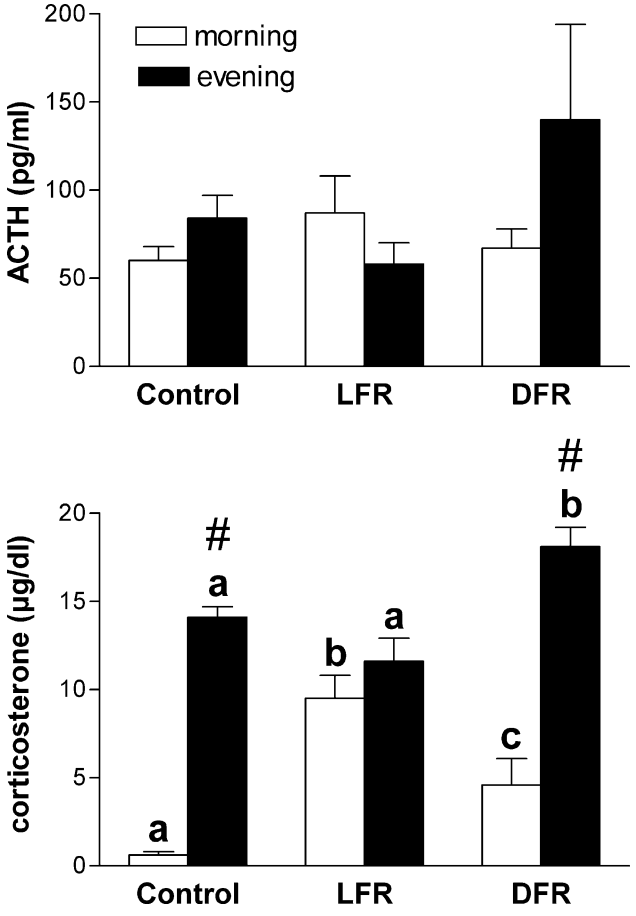

Fig. 2. Effect of two different chronic schedules of food restriction (FR), with morning (1100 h) or evening (1900 h) availability of food (LFR and DFR, respectively) on serum ACTH and corticosterone levels. Means and S.E.M. ($n=6-8$ per group for corticosterone, and $n=5-7$ for ACTH) corresponding to the different treatments. Open bars indicate morning and closed bars evening levels. Within the same time of day, groups labelled with different letters are statistically different (post hoc contrasts after the ANOVA); $^{\#}p<0.001$ versus corresponding morning levels.

1987; Morimoto et al., 1977). In DRF rats, increases in morning and evening levels of corticosterone were observed. Although we are not aware of any similar experiment in the literature, Garcia-Belenguer et al. (1993) observed a similar trend in rats given a 85% of the food eaten by controls just at lights off. Furthermore, although FR cannot be equated to food deprivation, the latter treatment has been found to reduce the plasmatic clearance rate of corticosterone, likely as a consequence of reduced hepatic metabolism of the hormone (Kiss et al., 1994; Woodward et al., 1991). Interestingly, DFR, despite increasing relative adrenal weight, did not modify absolute adrenal weight. In contrast, LFR increased both absolute and relative adrenal weights. Increases in relative adrenal weight have been repeatedly observed after FR (Garcia-Belenguer et al., 1993), but changes in absolute weight have not been presented. Whereas changes in relative adrenal weight may be the reflection of a greater resistance of adrenal, as compared to total body weight, to lose mass, the increase in absolute adrenal weight may be indicative of a chronic stress schedule.

None of the changes in adrenal weight or plasma corticosterone were accompanied by significant changes in ACTH levels. In fact, it has been previously found that neither changes in the daily rhythm of corticosterone nor changes associated to FR schedules are usually related to significant changes in circulating levels of ACTH using between-subject designs (Armario et al., 1987; Doell et al., 1981; Wilkinson et al., 1979, 1982), likely because daily food (or water) associated changes in adrenocortical secretion appear to be mainly related to increased adrenocortical responsiveness to ACTH (Dallman et al., 1978; Kaneko et al., 1980; Nicholson et al., 1985). It is still unclear whether this enhanced adrenal responsiveness to circulating ACTH is mediated by neural innervation of the adrenal, altered adrenal blood flow, or by other factors (Gibson and Krieger, 1981; Leal and Moreira, 1996; Ottenweller and Meier, 1982; Wilkinson et al., 1982). In accordance with ACTH data, no effect of FR schedules on CRF mRNA levels in the PVN was observed, either at lights on or at lights off. These data are in accordance with those reported in obese or lean Zucker rats submitted to a chronic schedule of 50% FR (Pesonen et al., 1992), but are in contrast to those reported Brady et al. (1990), who found a decrease in PVN CRF mRNA after a 14-day schedule of LFR. The reason for the discrepancies are unclear, but it should be noted that in the Brady et al.'s experiment the degree of FR was probably much higher than that imposed in the present experiment. It may be noted that the lack of differences between CRF mRNA levels at lights on and lights off is not surprising, as changes in CRF mRNA levels throughout the day are not in parallel with changes in circulating levels of corticosterone (Kwak et al., 1992, 1993). In addition, after several days of total food deprivation, increases in plasma corticosterone levels are associated to decreases in both anterior pituitary POMC and PVN CRF mRNAs (Bergendahl et al., 1992; Brady et al., 1990; Hwang and Guntz, 1997; Makino et al., 2001), which indicates that other factors are regulating adrenocortical activity during food deprivation and FR.

In conclusion, the present findings suggest that even moderated levels of chronic FR have an important impact on adrenocortical function, and that consequences of FR are partially different in function of the time of food availability. It is, nevertheless, suggested that it may be better to allow daily access to food at lights off because, otherwise, an apparent increase in the stressful properties of FR (increased relative adrenal weight), and more marked alteration of normal daily rhythms can occur.

Acknowledgments

This work was supported by grants from DGICYT (SAF2002-00623), CUR (1999SGR-330) and G03/005. We thank Dr. W.C. Engeland (Department of Surgery, University of Minnesota, Minneapolis, USA) for his gift of the ACTH antiserum.

References

Armario A, Castellanos JM. A simple procedure for direct corticosterone radioimmunoassay in the rat. Rev Esp Fisiol 1984;40:437–41.

Armario A, Montero JL, Jolin T. Chronic food restriction and the circadian rhythms of pituitary–adrenal hormones, growth hormone and thyroid-stimulating hormone. Ann Nutr Metab 1987;31:81–7.

Bergendahl M, Wiemann JN, Clifton DK, Huhtaniemi I, Steiner RA. Short-term starvation decreases POMC mRNA but does not alter GnRH mRNA in the brain of adult male rats. Neuroendocrinology 1992;56: 913–20.

Brady LS, Smith MA, Gold PW, Herkenham M. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 1990;52:441–7.

Dallman MF, Engeland WC, Rose JC, Wilkinson CW, Shinsako J, Siedenburg F. Nycthemeral rhythm in adrenal responsiveness to ACTH. Am J Physiol 1978;235:R210–8.

Doell RG, Dallman MF, Clayton RB, Gray GD, Levine S. Dissociation of adrenal corticosteroid production from ACTH in water-restricted female rats. Am J Physiol 1981;241:R21–4.

Garcia-Belenguer S, Oliver C, Mormede P. Facilitation and feedback in the hypothalamo–pituitary–adrenal axis during food restriction in rats. J Neuroendocrinol 1993;5:663–8.

Gibson MJ, Krieger DT. Circadian corticosterone rhythm and stress response in rats with adrenal autotransplants. Am J Physiol 1981; 240(4):E363–6.

Goeders NE, Guerin GF. Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology (Berl) 1994;114:63–70.

Honma KI, Honma S, Hiroshige T. Critical role of food amount for prefeeding corticosterone peak in rats. Am J Physiol 1983;245: R339–44.

Hwang BH, Guntz JM. Downregulation of corticotropin-releasing factor mRNA, but not vasopressin mRNA, in the paraventricular hypothalamic nucleus of rats following nutritional stress. Brain Res Bull 1997; 43:509–14.

Kaneko M, Hiroshige T, Shinsako J, Dallman MF. Diurnal changes in amplification of hormone rhythms in the adrenocortical system. Am J Physiol 1980;239:R309–16.

Kantak KM, Black Y, Valencia E, Green-Jordan K, Eichenbaum HB. Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. J Neurosci 2002;22:1126–36.

Kiss A, Jezova D, Aguilera G. Activity of the hypothalamic pituitary adrenal axis and sympathoadrenal system during food and water deprivation in the rat. Brain Res 1994;663:84–92.

Krieger DT. Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. Endocrinology 1974;95: 1195–201.

Krieger DT, Hauser H, Krey LC. Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. Science 1977;197:398–9.

Kwak SP, Young EA, Morano I, Watson SJ, Akil H. Diurnal corticotropin-releasing hormone mRNA variation in the hypothalamus exhibits a rhythm distinct from that of plasma corticosterone. Neuroendocrinology 1992;55:74–83.

Kwak SP, Morano MI, Young EA, Watson SJ, Akil H. Diurnal CRH mRNA rhythm in the hypothalamus: decreased expression in the evening is not dependent on endogenous glucocorticoids. Neuroendocrinology 1993; 57:96–105.

Leal AM, Moreira AC. Feeding and the diurnal variation of the hypothalamic–pituitary–adrenal axis and its responses to CRH and ACTH in rats. Neuroendocrinology 1996;64:14–9.

Makino S, Kaneda T, Nishiyama M, Asaba K, Hashimoto K. Lack of decrease in hypothalamic and hippocampal glucocorticoid receptor mRNA during starvation. Neuroendocrinology 2001;74:120–8.

Marti O, Gavalda A, Marti J, Gil M, Giralt M, Lopez-Calderon A, et al. Chronic stress induced changes in LH secretion: the contribution of anorexia associated to stress. Life Sci 1993;52:1187–94.

Marti O, Marti J, Armario A. Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. Physiol Behav 1994;747–53.

Merlo-Pich E, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R, Chiamulera C. Common neural substrates for the addictive properties of nicotine and cocaine. Science 1997;275:83–6.

Moberg GP, Bellinger LL, Mendel VE. Effect of meal feeding on daily rhythms of plasma corticosterone and growth hormone in the rat. Neuroendocrinology 1975;19:160–9.

Morimoto Y, Arisue K, Yamamura Y. Relationship between circadian rhythm of food intake and that of plasma corticosterone and effect of food restriction on circadian adrenocortical rhythm in the rat. Neuroendocrinology 1977;23:212–22.

Nicholson S, Lin JH, Mahmoud S, Campbell E, Gillham B, Jones M. Diurnal variations in responsiveness of the hypothalamo–pituitary–adrenocortical axis of the rat. Neuroendocrinology 1985;40:217–24.

Ottenweller JE, Meier AH. Adrenal innervation may be an extrapituitary mechanism able to regulate adrenocortical rhythmicity in rats. Endocrinology 1982;111:1334–8.

Pesonen U, Huupponen R, Rouru J, Koulu M. Hypothalamic neuropeptide expression after food restriction in Zucker rats: evidence of persistent neuropeptide Y gene activation. Brain Res Mol Brain Res 1992;16: 255–60.

Simmons DM, Arriza JL, Swanson LW. A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radiolabeled single-stranded RNA probes. J Histotechnol 1989;12:169–81.

Stephan FK. Phase shifts of circadian rhythms in activity entrained to food access. Physiol Behav 1984;32:663–71.

Wilkinson CW, Shinsako J, Dallman MF. Daily rhythms in adrenal responsiveness to adrenocorticotropin are determined primarily by the time of feeding in the rat. Endocrinology 1979;104:350–9.

Wilkinson CW, Shinsako J, Dallman MF. Rapid decreases in adrenal and plasma corticosterone concentrations after drinking are not mediated by changes in plasma adrenocorticotropin concentration. Endocrinology 1982;110:1599–606.

Woodward CJ, Hervey GR, Oakey RE, Whitaker EM. The effects of fasting on plasma corticosterone kinetics in rats. Br J Nutr 1991;66: 117–27.

Xu B, Kalra PS, Farmerie WG, Kalra SP. Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. Endocrinology 1999;140:2868–75.