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Stress differentially regulates brain expression of corticotropin-releasing factor in binge-like eating prone and resistant female rats

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ABSTRACT

The expression of corticotropin-releasing factor (CRF), a neuropeptide that regulates endocrine and behavioral responses to stress, was assessed in the brain in rats prone or resistant to stress-induced binge-like eating of sucrose. Female Sprague-Dawley rats were subjected to unpredictable intermittent 1-h access to sucrose in non-stressful conditions or after exposure to three foot shock stress sessions. Experimental sessions were performed at metestrus, diestrus, and proestrus. The rats were assigned to the binge-like eating prone (BEP) or the binge-like eating resistant (BER) phenotypes according to the rats' persistently high or low sucrose intake following three stress sessions. The BEP rats displayed elevated consumption of sucrose in non-stressful conditions and an additional significant increase in sucrose intake in response to stress. Conversely, the BER rats showed lower sucrose intake in non-stressful conditions, and stress did not increase sucrose intake in this phenotype. The brain expression of CRF mRNA and plasma corticosterone levels were assessed 30 min after the last stress session at the diestrous phase of the estrous cycle. Stress triggered a significant increase in plasma corticosterone levels and strongly increased CRF mRNA expression in the paraventricular hypothalamic nucleus in the BER but not in the BEP rats. However, the BEP but not the BER rats demonstrated a significant increase in CRF mRNA expression in the bed nucleus of the stria terminalis (BNST) after stress. Hyporeactivity of the hypothalamic-pituitary-adrenal axis and the higher CRF expression in the BNST in BEP rats may contribute to stress-induced binge-like sucrose eating in the BEP phenotype.

1. Introduction

Binge eating is characterized by consumption of an unusually excessive amount of food within a discrete period of time with loss of control over eating (APA., 2013). Binge eating is a core symptom of many eating disorders such as binge eating disorder, bulimia nervosa, and the binge/purge subtype of anorexia nervosa (APA., 2013). Negative mood and life stress events are among the most common antecedents of binge eating episodes (Berg et al., 2015; Cattanach, Malley, & Rodin, 1988; Lingswiler, Crowther, & Stephens, 1987; Smyth et al., 2007). During bingeing episodes, highly palatable foods are frequently eaten in an attempt to cope with stress (Dallman, Pecoraro, & la Fleur, 2005; Polivy & Herman, 1999; Rutters, Nieuwenhuizen, Lemmens, Born, & Westerterp-Plantenga, 2009; Ulrich-Lai, 2016; Zellner et al., 2006). A large variability exists in the propensity to develop binge eating. It is common to be frequently exposed to stress in everyday life and to have access to palatable high-energy food; however, only a small proportion of the general population develops binge eating symptoms (Bulik, Sullivan, & Kendler, 1998; Reichborn-Kjennerud, Bulik, Tambs, & Harris, 2004). The propensity for binge eating depends on individual differences based on genetic and environmental factors.

Our model of binge-like eating of sucrose in female rats is based on individual sensitivity to increase sucrose intake in response to stress (Calvez et al., 2016; Calvez & Timofeeva, 2016). To develop

Abbreviations: ACTH, adrenocorticotropic hormone; BEP, binge-like eating prone; BER, binge-like eating resistant; BNST, bed nucleus of the stria terminalis; BNSTav, anteroventral nucleus of the BNST; BNSTov, oval nucleus of the BNST; CeA, central amygdala; CRF, corticotropin-releasing factor; CRF_{1R}, CRF type 1 receptor; CRF_{2R}, CRF type 2 receptor; HPA, hypothalamic-pituitary-adrenal; icv, intracerebroventricular; MPOA, medial preoptic area; NI, nucleus incertus; OD, optical density; PVN, paraventricular hypothalamic nucleus; PVNp, parvocellular part of the PVN; RXFP3, relaxin family peptide receptor 3.

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this model, we subjected female rats to intermittent unpredictable access to sucrose and repeated foot shock stress. The binge-like eating prone (BEP) and binge-like eating resistant (BER) phenotypes were selected based on persistently higher or lower sucrose intake after repeated unpredictable episodes of stress. The BEP rats displayed higher sucrose consumption in non-stressful conditions than the BER rats, and the BEP rats' sucrose intake further increased under stressful conditions. Furthermore, the BEP rats displayed several behaviors similar to clinical features of binge eating, such as increased sucrose intake even when not deprived of chow (similar to eating when not physically hungry), increased sucrose intake in an aversively bright chamber (similar to compulsive eating), and increased initial rate of sucrose intake (similar to increased reward sensitivity) (Banca, Harrison, & Voon, 2016; Calvez & Timofeeva, 2016; Rosenberg et al., 2013; Schienle, Schafer, Hermann, & Vaitl, 2009). In addition, the plasma corticosterone levels showed lower reactivity to stress in the BEP rats compared to the BER phenotype (Calvez & Timofeeva, 2016).

It has been previously shown in humans and animals that intake of palatable food reduces the magnitude of the stress response (Foster et al., 2009; Kinzig, Hargrave, & Honors, 2008; la Fleur, Houshyar, Roy, & Dallman, 2005; Markus, Panhuysen, Tuiten, & Koppeschaar, 2000; Martin & Timofeeva, 2010; Polivy & Herman, 1999; Tryon et al., 2015; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2007). In fact, plasma corticosterone and adrenocorticotropic hormone (ACTH) levels and hypothalamic expression of corticotropin-releasing factor (CRF) were not increased after exposure to stress in rats with access to palatable food such as sucrose and/or lard (Foster et al., 2009; Martin & Timofeeva, 2010; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2007). Free access to palatable food also decreased the expression of CRF in the bed nucleus of the stria terminalis (BNST) but increased the levels of CRF transcript in the central nucleus of amygdala (CeA) and did not affect expression of CRF mRNA in the medial preoptic area (MPOA) (Foster et al., 2009). The blunting effect of palatable food on stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis is considered an attempt to reduce the negative effects induced by stress with hedonic "self-medication" (Dallman et al., 2003; Ulrich-Lai, 2016).

In our model of binge-like eating, plasma corticosterone responses to stress differed in BEP and BER rats (Calvez & Timofeeva, 2016). Before exposure to repeated stress and intermittent access to sucrose, the BEP rats displayed a reduced corticosterone response to the first exposure to stress compared to the BER rats. Furthermore, after repeated stress and intermittent access to sucrose, the corticosterone responses to stress were completely inhibited in the BEP phenotype (Calvez & Timofeeva, 2016). CRF expressed in the parvocellular PVN is a critical regulator of the responses of the HPA axis to stress (Richard & Timofeeva, 2010; Sawchenko, Li, & Ericsson, 2000). Hypothalamic and extrahypothalamic CRF is also implicated in behavioral stress responses such as stress-induced anxiety and regulation of appetite (Anthony et al., 2014; Koob & Heinrichs, 1999; Mitra, Lenglos, & Timofeeva, 2015). In rodents, the hypothalamic and extrahypothalamic CRF systems are involved in the effects of stress on drug-seeking (Koob, 2009) and palatable food intake (Cottone et al., 2009; Foster et al., 2009; Lenglos, Mitra, Guevremont, & Timofeeva, 2013; Micioni Di Bonaventura et al., 2014; Pucci et al., 2015). In a model of binge-like eating of palatable food induced by frustration stress in female rats submitted to repeated food restriction, CRF expression was upregulated in the hypothalamus and in the amygdala complex under stressful conditions (Pucci et al., 2015). In this model, stress-induced binge-like eating was suppressed by administration of the CRF receptor antagonist in the BNST (Micioni Di Bonaventura et al., 2014) supporting the idea that the hypothalamic and extrahypothalamic CRF systems are important in mediating stress effects on eating behavior. Our model of stress-induced binge-like eating of sucrose does not require repeated food restriction for expression of binge-like eating episodes but is based on individual sensitivity to reward and stress. Given the important role of CRF in stress response and food intake regulation, we hypothesized that the BER and BEP phenotypes would have differential profiles of activity of the CRF systems in the hypothalamus and in extrahypothalamic nuclei involved in regulation of eating and stress response. The present study was designed to explore the CRF system by comparing CRF mRNA expression in the PVN, BNST, CeA, and MPOA in BEP and BER female rats in non-stressful and stressful conditions.

2. Materials and methods

2.1. Animals

Young female Sprague Dawley rats (PD 36–39; n = 41) were obtained from the Canadian Breeding Laboratories (St-Constant, QC, Canada). Rats were housed in individual plastic cages lined with wood shavings, maintained on a 12:12 h dark-light cycle, and fed standard laboratory rat chow (2018 Teklad Global 18% Protein Rodent Diet; 3.1 kcal/g, Harlan Teklad, Montreal, QC, Canada) and tap water *ad libitum*, unless otherwise specified. All rats were cared for and handled according to the Canadian Guide for the Care and Use of Laboratory Animals, and the present protocol was approved by the host institutional animal care committee.

2.2. Binge-like eating classification

Rats were classified as binge-like eating prone rats (BEP) and binge-like eating resistant rats (BER) based on consistently higher (for BEP) or lower (for BER) sucrose intake after 3 unpredictable foot-shock stress sessions as previously described (Calvez et al., 2016; Calvez & Timofeeva, 2016). First, the rats were given intermittent access in their home cages to a solution of 10% sucrose (0.4 kcal/ml, in a bottle) for 1 h at the beginning of the dark cycle with intervals of 1–3 days between each access session. After stabilization of sucrose intake, three 1-h sucrose access sessions in home cages in non-stressful conditions were performed with intervals of 3–4 days between sessions (Fig. 1A). Thereafter the rats were exposed to 3 foot-shock stress sessions immediately before 1-h access to sucrose. The stress sessions were performed in a separate room and consisted of four 0.6 mA DC impulses of 3-s duration with 15-s inter-shock intervals delivered to the grid floor of a foot-shock apparatus. Immediately after stress, the rats were returned to their home cages where they had an access to sucrose for 1 h. An additional session of sucrose access without stress was carried out in between the stress sessions to avoid a learned association of stress and sucrose access. Sucrose intake after each stress session was divided into low, intermediate and high intake tertiles. On average, the low intake was between 2.8 and 5.1 kcal, the intermediate intake was between 5.1 and 6.3 kcal and the high intake was between 6.3 and 10.0 kcal. For inclusion in the BEP group, a rat could consume either high amounts of sucrose after 3 stress sessions or high amounts of sucrose after 2 stress sessions and an intermediate amount of sucrose after 1 stress session (Fig. 1C). The same procedure was used to classify the BER rats but with low intake. According to this procedure, about 30% of the rats were classified as BEP (n = 12), and about 30% were classified as BER (n = 12). The other rats (about 40%), with inconsistent sucrose intake across the stress sessions, were excluded from further analyses.

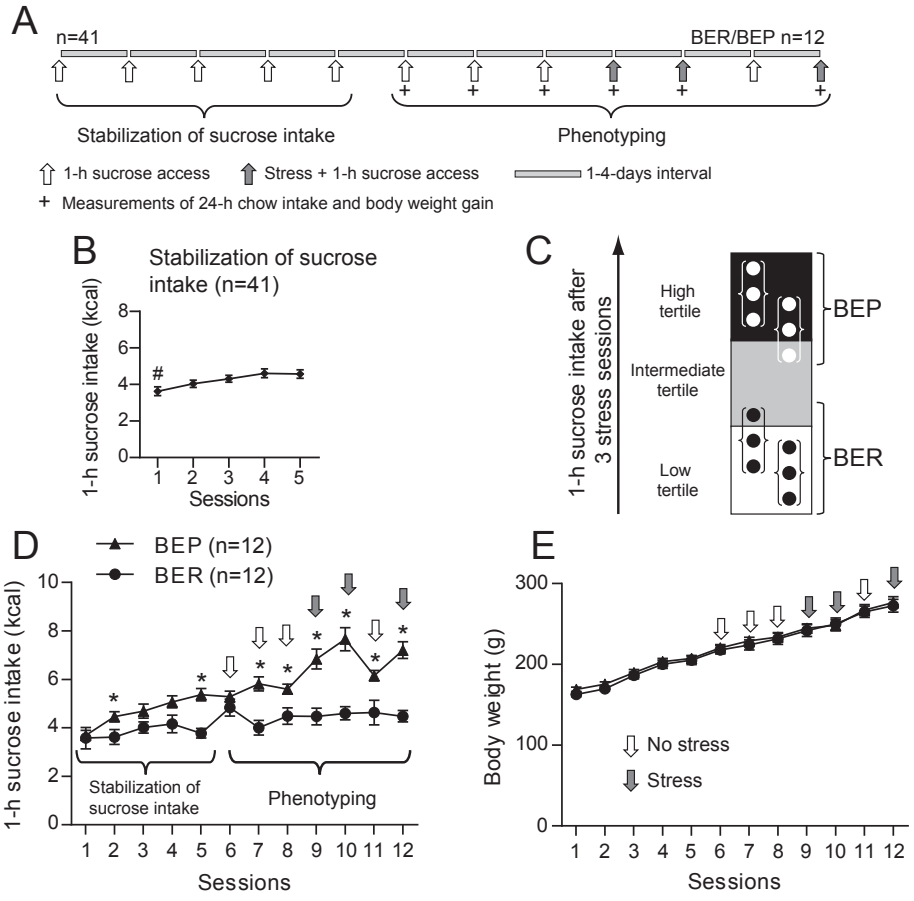

Fig. 1. (A) Diagram of the experimental procedure. Rats were first given 1-h intermittent access to 10-% sucrose in their home cages at the beginning of the dark phase. After stabilization of sucrose intake, three 1-h access sessions to sucrose were performed in the non-stressful conditions or after three foot shock stress and were used for rats' phenotyping. An additional session of sucrose access without stress was carried out in between the stress sessions to avoid a learned association of stress and sucrose access. (B) One-hour sucrose intake for all of the rats (n = 41) during the initial 1st–5th access sessions that were used to assess stabilization of sucrose intake. (C) Rats were classified as BER or BEP phenotypes based on the consistency of low or high intake of sucrose after the 3 stress sessions. A rat was included to the BEP group if it consumed either large amounts of sucrose after three stress sessions or large amounts of sucrose after two stress sessions and an intermediate amount after one stress session. A rat was included to the BER group if it consumed either low amounts of sucrose after three stress sessions or low amounts of sucrose after two stress sessions and an intermediate amount after one stress session. (D) 1-h sucrose intake and (E) body weight for all the experimental sessions in BER (n = 12) and BEP (n = 12) rats. *Significantly ($p < 0.05$) different compared to BER rats for the same session. #Significant ($p < 0.05$) difference between sucrose intake during the 1st session compared to the 4th and 5th sessions.

2.3. Sucrose and chow intake and body weight in BER and BEP rats

After initial 5 sessions of 1-h sucrose intake that were used to assess the stabilization of sucrose intake, the average 1-h sucrose intake, 24-h chow intake, and 24-h body weight gain were assessed after three 1-h sessions of sucrose access in non-stressful conditions (6th, 7th, and 8th sessions) and after 3 stress sessions (Fig. 1A). Body weight and chow intake was measured daily 2 h before the beginning of the dark cycle. Vaginal smears were collected daily 3 h before the dark phase and then stained with methylene blue (Sigma, Oakville, ON, Canada). The phases of the estrous cycle were determined as previously described (Lenglos, Calvez, & Timofeeva, 2015) and the stress and sucrose access sessions were performed at metestrus, diestrus, and proestrus. A previous study (Calvez & Timofeeva, 2016) has shown that the intake of sucrose and chow was not different between non-estrous days of the estrous cycle. In the present experiment, stress and sucrose access sessions were done only on metestrus, diestrus, and proestrus days of the estrous cycle.

2.4. Plasma corticosterone determination

At the end of the experiment, the rats at the diestrous phase of estrous cycle were euthanized 30 min after a foot-shock stress session (n = 6/group) or in a non-stressful condition (n = 6/group) at the beginning of the dark phase. Immediately after stress, the rats were transferred to their home cages where all rats had no access to chow or sucrose during 30 min before euthanasia. The rats were rapidly anesthetized (60 mg/kg ketamine plus 7.5 mg/kg xylazine), and intracardial blood was collected. The rats were then perfused intracardially with 200 ml of saline followed by 500 ml of paraformaldehyde (4%) solution. Plasma corticosterone was determined in blood samples in duplicate with the Corticosterone ELISA kit for rats and mice (sensitivity, 4 pg/ml; interassay coefficient of variation, 6.2%; Cayman Chemical, Ann Arbor, MI).

2.5. In situ hybridization for CRF mRNA expression

Brain sections were prepared as previously described (Mitra et al., 2015; Poulin & Timofeeva, 2008). Briefly, the rat brains were removed at the end of the perfusion and post-fixed in paraformaldehyde for 1 week. They were then transferred to a solution containing paraformaldehyde (4%) and sucrose (20%) before being cut 12 h later using a sliding microtome (Histoslide 2000, Heidelberger, Germany). Thirty-micron-thick sections were collected and stored at −30 °C in a cold sterile cryoprotecting solution

containing sodium phosphate buffer (50 mM), ethylene glycol (30%), and glycerol (20%). The brains were then processed for analyses of CRF mRNA expression using *in situ* hybridization as previously described (Lenglos et al., 2015). The brain sections were mounted on poly-L-lysine-coated slides and were fixed for 20 min in paraformaldehyde (4%), digested for 25 min at 37 °C with proteinase K (10 μg/ml), acetylated with acetic anhydride (0.25%), and dehydrated through graded ethanol concentrations. Sections were incubated overnight with antisense ^{35}S-labeled cRNA probes (10^7 cpm/ml) of CRF (generated from the 1063-bp fragment of rat CRF cDNA; GenBank accession number NM_031019; Dr. K. Mayo, Northwestern University, Evanston, IL) at 60 °C. Thereafter, the slides were rinsed with sodium chloride-sodium citrate solution, digested with ribonuclease-A (20 μg/ml), washed in descending concentrations of sodium chloride-sodium citrate solution, and dehydrated through an ethanol gradient. The slides were defatted in toluene, dipped in nuclear emulsion (Eastman Kodak, Rochester, NY), and exposed for 1 week before being developed. Finally, tissues were counterstained with thionin, dehydrated through graded ethanol concentrations, cleared in toluene, and cover-slipped with mounting medium.

The slides were examined with dark-field microscopy using an Olympus BX61 microscope (Olympus Canada, Richmond Hill, ON, Canada). Images were acquired with a DVC-2000C digital camera (DVC Company Inc., Austin, TX) and analyzed with Stereo Investigator software (MBF Bioscience, Williston, VT). The system was calibrated for each set of analyses to prevent saturation of the integrated signal. CRF mRNA expression was analyzed in the parvocellular part of the PVN (PVNp; 1.6–1.9 mm caudal to the bregma), the oval and anteroventral nuclei of the BNST (the BNSTov and the BNSTav respectively, from 0.1 mm rostral to 0.4 mm caudal to the bregma), the MPOA (0.2–0.6 mm caudal to the bregma) and the CeA (1.9–2.4 mm caudal to the bregma). The brain sections of each rat were matched for rostrocaudal levels as closely as possible, and the accuracy of outlining of the brain structures was verified using bright-field illumination of thionin counterstaining. Mean optical density (OD) was obtained by measuring the OD of pixels of the positive hybridization signal on 2–4 sections depending on the region of interest and subtracting background readings taken from the areas immediately surrounding the analyzed region. The individual score for each rat was normalized to the mean value of the BER non-stressed group to obtain the relative levels of mRNA expression. The normalized scores of the rats were then averaged across the experimental groups. Additionally, we calculated the change of CRF expression after stress relatively to the non-stressful condition within each phenotype.

2.6. Statistical analyses

Results are presented as mean values ± standard errors of the mean. Repeated 1-way ANOVA followed by Tukey's multiple comparisons test was used to assess stabilization of sucrose intake over 1st–5th sessions. Comparison of 1-h sucrose intake and body weight between the BER and BEP phenotypes for each session was performed using unpaired *t*-test. Repeated 2-way ANOVA was used to detect the main and interactive effects of phenotype (BEP *vs* BER) and stress (No stress *vs* Stress) on sucrose, chow, and total energy intake and on body weight gain as well as on the expression of CRF mRNA in the brain and on the plasma corticosterone level. *Post-hoc* comparisons between the groups were performed using Tukey' multiple comparisons test when the main or interactive ANOVA effects were significant. Unpaired *t*-test was used to compare the relative changes in plasma corticosterone and CRF mRNA expression after stress in BER and BEP groups. Results were considered significant with p values < 0.05. Statistical analyses were performed using Prism 6.04 (GraphPad Software Inc., La Jolla, CA).

3. Results

3.1. Determination of BER and BEP phenotypes

Before phenotyping procedure, stabilization of sucrose intake was assessed in all of the rats with intermittent (1–3-days interval between sessions) 1-h access to sucrose. The sucrose intake at the 1st access was significantly lower compared to the 4th ($p = 0.0210$) and 5th ($p = 0.0299$) accesses (Fig. 1B). However, after the 2nd assess, the 1-h sucrose intake was not significantly different ($p > 0.05$) compared to the 3rd, 4th, and 5th sessions. In the initial population of 41 rats, 12 rats were characterized as BER, and 12 rats were characterized as BEP according to their consistently low or high sucrose intake after 3 stress sessions (Fig. 1B). The BEP rats consumed significantly higher amount of sucrose than BER rats during the 2nd ($p = 0.0377$) and 5th ($p < 0.0001$) sessions and during the 7th-12th ($p = 0.0003$, $p = 0.0115$, $p = 0.0003$, $p < 0.0001$, $p = 0.0135$, $p < 0.0001$, respectively) sessions (Fig. 1D). Conversely, body weights (Fig. 1E) were not significantly different ($p > 0.05$) between the phenotypes for any session.

Two-way ANOVA revealed the significant effect of phenotype ($F_{1,22} = 49.99$, $p < 0.0001$) and stress ($F_{1,22} = 15.16$, $p = 0.0011$) as well as a significant interactive effect of stress and phenotype ($F_{1,22} = 10.18$, $p = 0.0051$) on 1-h sucrose intake in non-stressful (6th–8th access sessions) and stressful (after 3 stress sessions) conditions (Fig. 2). The BEP rats consumed 34% more sucrose solution in the non-stressful condition ($p = 0.0015$) and 62% more sucrose after exposure to stress ($p < 0.0001$) when compared to the BER rats. Additionally, sucrose intake was significantly increased after stress in the BEP rats (+25%, $p = 0.0016$) but not in the BER rats ($p > 0.05$) (Fig. 2).

Neither phenotype ($F_{1,22} = 2.068$, $p > 0.05$) nor the interactive effect of stress and phenotype ($F_{1,22} = 0.0138$, $p > 0.05$) had a significant effect on chow intake. Chow intake was significantly reduced after exposure to stress in BER rats ($p = 0.0154$) whereas a decrease in chow intake did not reach statistical significance in BEP rats ($p > 0.05$) (Table 1). Accordingly, the 24-h total energy intake (chow and sucrose intake) was significantly reduced after the stress sessions only in the BER rats ($p = 0.0316$). Significant effects of stress and phenotype on the total energy intake were found

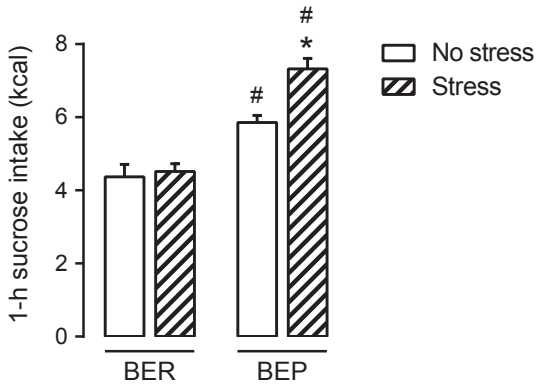

Fig. 2. One-hour sucrose intake under non-stressful condition (average sucrose intake during 3 sucrose access sessions after stabilization of sucrose intake) and after exposure to foot shock stress (average sucrose intake after the 3 stress sessions). *Significantly ($p < 0.05$) different from the non-stressful condition within the same phenotype; #Significantly ($p < 0.05$) different from BER rats within the same stressful condition. BEP – binge-like eating prone rats ($n = 12$); BER – binge-like eating resistant rats ($n = 12$).

Table 1
Twenty-four-h chow intake, total energy intake and body weight gain in binge-like eating resistant (BER) and binge-like eating prone (BEP) rats in the non-stressful conditions and after stress.

	BER		BEP	
	No stress	Stress	No stress	Stress
24-h chow intake (kcal)	62.7 ± 2.7	56.1 ± 2.1[a]	65.0 ± 2.2	59.6 ± 1.6
24-h total energy intake (kcal)	67.2 ± 3.0	59.4 ± 1.9[a]	71.1 ± 2.5	67.0 ± 1.8
24-h body weight gain (g)	3.6 ± 0.6	0.7 ± 0.5[a]	2.5 ± 0.5	2.5 ± 0.3[b]

[a] Significantly different from the non-stressful condition within the same phenotype.
[b] Significantly different from BER rats within the same stressful condition.

($F_{1,22} = 15.49$, $p = 0.0015$ and $F_{1,22} = 5.485$, $p = 0.0345$, respectively). As a consequence, body weight gain was significantly reduced after the stress sessions in the BER rats ($p = 0.0007$) and not in the BEP rats ($p > 0.05$) (Table 1). The 2-way ANOVA revealed a significant effect of the interaction of stress and phenotype ($F_{1,22} = 14.70$, $p = 0.0012$) on body weight gain. A decrease in body weight gain in the BER rats after exposure to stress was transient and compensated for during non-stressful days, resulting in comparable body weights in the phenotypes at the beginning (162.7 ± 2.9 g for the BER rats and 169.1 ± 2.7 g for the BEP rats) and the end of experiment (272.6 ± 7.9 g for the BER rats and 276.7 ± 7.1 g for the BEP rats) (Fig. 1E). Only time had a significant effect on body weight regardless of rats' phenotypes as was revealed by ANOVA ($F_{1,22} = 887.9$, $p < 0.0001$).

3.2. Plasma corticosterone and CRF mRNA expression in the PVN

The activity of the HPA axis was evaluated by determination of the levels of plasma corticosterone and CRF expression in the parvocellular part of the PVN in non-stressful conditions and 30 min after the stress sessions in the BER and BEP rats (Fig. 3). A significant interaction of phenotype and stress on the plasma corticosterone levels (Fig. 3A) was revealed by ANOVA ($F_{1,19} = 8.338$, $p = 0.0098$). In the BER rats, stress induced a significant increase in plasma corticosterone ($p = 0.0359$) whereas no difference was found between the non-stressful and stressful conditions in the BEP rats ($p > 0.05$).

Consequently, after the stress sessions, the BER rats displayed a significantly higher plasma corticosterone levels than the BEP rats ($p = 0.0431$) and the relative change of plasma corticosterone after stress was greater in BER rats than BEP rats ($p = 0.0058$, Table 2). Similarly, a significant interactive effect of phenotype and stress was found on CRF mRNA expression in the PVNp ($F_{1,19} = 5.679$, $p = 0.0292$, Fig. 3B, C). The expression of CRF mRNA in the PVNp rats was significantly increased after stress in BER ($p = 0.0481$) but not in BEP rats ($p > 0.05$). Accordingly, stress induced a significantly higher increase of CRF mRNA expression in BER rats compared to the BEP phenotype ($p = 0.0188$, Table 2).

3.3. CRF mRNA expression in the BNST

The expression of CRF mRNA was determined in the oval and anteroventral nuclei of the BNST in the BER and BEP rats (Fig. 4). In the BNSTov and the BNSTav, significant effects of phenotype (BNSTov, $F_{1,19} = 11.82$, $p = 0.0028$; BNSTav, $F_{1,19} = 11.32$, $p = 0.0033$) and stress (BNSTov, $F_{1,19} = 9.438$, $p = 0.0063$; BNSTav, $F_{1,19} = 10.31$, $p = 0.0046$) were revealed by the ANOVA. A significant increase in CRF mRNA expression after stress was observed in BEP rats in the BNSTov ($p = 0.0267$) and the BNSTav ($p = 0.0182$). In BER rats, CRF mRNA expression was slightly increased in the BNSTov and the BNSTav after stress, but these increases did not reach statistical significance ($p > 0.05$). Consequently, CRF expression in the BNSTov and the BNSTav was significantly higher after stress in BEP rats when compared to BER rats ($p = 0.0212$ and $p = 0.0201$, respectively). However, despite a higher increase of CRF mRNA expression in the BNST of BEP rats, the relative changes were not different between the 2 groups ($p > 0.05$, Table 2).

3.4. CRF mRNA expression in the MPOA and the CeA

Neither stress ($F_{1,19} = 0.2586$, $p > 0.05$) nor the interactive effects of stress and phenotype ($F_{1,19} = 1.511$, $p > 0.05$) had the significant effects on CRF mRNA expression in the MPOA (Fig. 5A, C); however, a significant effect of phenotype was detected ($F_{1,19} = 12.78$, $p = 0.0025$). *Post-hoc* comparisons revealed that after stress, BEP rats displayed significantly higher CRF mRNA expression in the MPOA than BER rats ($p = 0.024$).

No significant effects of stress ($F_{1,20} = 0.7470$, $p > 0.05$), phenotype ($F_{1,20} = 2.547$, $p > 0.05$), nor interactive effects of both factors ($F_{1,20} = 0.1692$, $p > 0.05$) were found on CRF mRNA expression in the CeA of BER and BEP rats (Fig. 5B).

4. Discussion

This study reports for the first time a differential pattern of expression of CRF in brain regions involved in stress and food intake regulation in female rats prone and resistant to stress-induced binge-like sucrose intake. In response to stress, the BER rats significantly enhanced expression of CRF mRNA in the PVN and strongly increased the plasma corticosterone levels. Conversely, the BEP rats displayed blunted stress-induced activation of the HPA axis with no detectable increase in plasma corticosterone levels and CRF mRNA expression in the PVN. In contrast, CRF expression in the BNST was significantly higher after stress in BEP rats but not in BER rats. Hyporeactivity of the HPA axis and the higher CRF expression in the BNST in BEP rats may contribute to stress-induced binge-like sucrose eating in the BEP phenotype.

The model of binge-like eating used in this study was based on the individual sensitivity of young adult female SD rats to stress and sucrose that resulted in increased sucrose consumption in response to stress. According to previous studies (Calvez et al., 2016; Calvez & Timofeeva, 2016), BEP rats consumed a larger amount of sucrose compared to BER rats in non-stressful conditions (34% > BER), and the BEP rats' sucrose intake increased significantly (64% > BER) under stress. Conversely, stress did not affect sucrose intake in BER rats but decreased total energy intake (sum of chow and sucrose intake) in this phenotype, resulting in lower body weight gain over 24 h following stress in BER rats. However, the BER rats compensated for their lower energy intake after stress during the non-stressful days, and the rats' body weight did not differ between the phenotypes from the beginning to the end of experiment. In the present study the measurements of sucrose and chow intake were performed at the non-estrous days and the detection of expression of CRF mRNA was estimated during the diestrus phase. The levels of ovarian hormones, estradiol and progesterone, are increased at the proestrus phase and affect CRF activity during proestrus (Hiroshige,

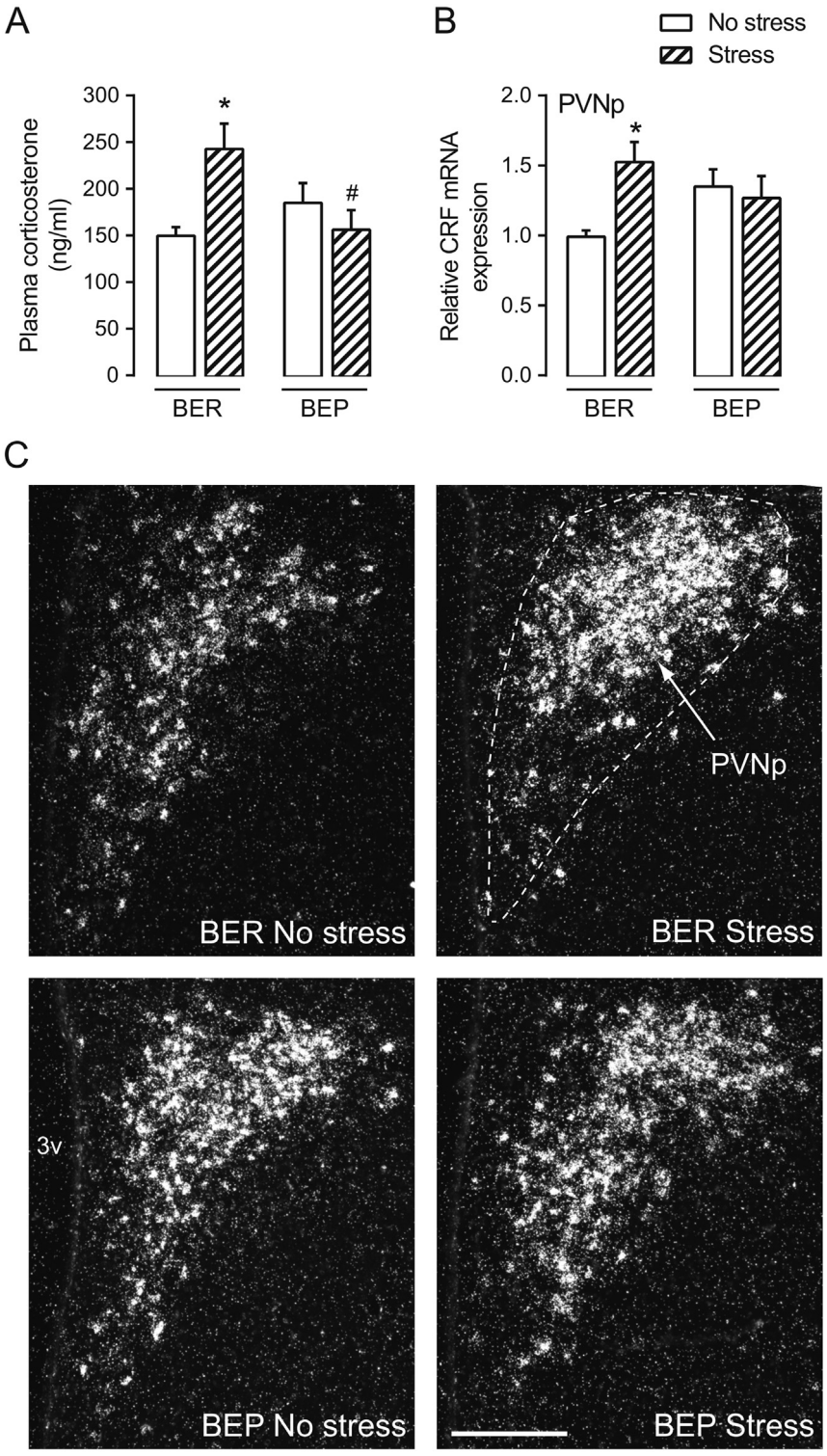

Fig. 3. Plasma corticosterone levels (A) and relative levels of CRF mRNA expression in the parvocellular part of the paraventricular nucleus of the hypothalamus (PVNp, B) in BEP and BER rats under non-stressful conditions and after exposure to foot-shock stress. (C) Darkfield micrographs illustrating positive hybridization signal for CRF mRNA in the PVNp under non-stressful conditions and following exposure to foot-shock stress in BEP and BER rats. *Significantly ($p < 0.05$) different from the non-stressful condition within the same phenotype; #Significantly ($p < 0.05$) different from BER rats within the same stressful condition. N = 6 rats/group. 3v – third ventricle; BEP, binge-like eating prone rats; BER, binge-like eating resistant rats. Scale bar, 200 μm.

Abe, Wada, & Kaneko, 1973; Nappi & Rivest, 1995) and food intake during estrus (Asarian & Geary, 2006, 2013). Ovarian hormones induced tonic but not cyclic inhibition of feeding in a binge-like eating rat model developed by intermittent access to dietary fat (Yu, Geary, & Corwin, 2008). Conversely, bingeing episodes triggered by frustration stress in repeatedly food-restricted rats were sensitive to the estrous phase (Micioni Di Bonaventura, Ciccocioppo, Massi, & Cifani, 2013). Future experiments should

Table 2
Percentage of changes in the levels of corticosterone and CRF mRNA expression at stress relative to the non-stressful condition in binge-like eating resistant (BER) and binge-like eating prone (BEP) rats.

	BER	BEP
Plasma corticosterone	+50.0% ± 14.6%[a]	−15.4% ± 11.3%[b]
CRF mRNA in the		
PVN	+39.3% ± 10.5%[a]	−6.0% ± 11.5%[b]
BNSTov	+28.7% ± 17.0%	+67.0% ± 17.4%[a]
BNSTav	+25.3% ± 14.4%	+61.4% ± 15.7%[a]
MPOA	−14.5% ± 4.0%	+23.8% ± 24.6%
CeA	+4.7% ± 9.3%	+13.3% ± 14.2%

[a] Significantly ($p < 0.05$) different from the non-stressful condition within each phenotype.
[b] Significantly ($p < 0.05$) different from BER rats.

investigate the impact of the estrous phase on binge eating episodes and CRF expression in the present model of binge eating.

Our previous study has demonstrated that the individual sensitivity to binge-like eating in our model may depend on innate abnormal reward sensitivity and altered reactivity of the HPA axis to stress (Calvez & Timofeeva, 2016). However, variability in fear and freezing responses to foot shock stress (Shumake, Furgeson-Moreira, & Monfils, 2014) may also contribute to the development of binge-like phenotype. Using another type of stress in this model may help to distinguish the relative role of these factors.

Previous experiments have shown that before exposure to sucrose and stress, the BEP rats displayed a reduced corticosterone response to stress whereas intermittent access to sucrose and unpredictable stress sessions led to the complete inhibition of stress-evoked corticosterone in this phenotype (Calvez & Timofeeva, 2016). Given the role of HPA axis activity in binge eating in humans (Timofeeva & Calvez, 2014) and the recent evidence that implicates CRF in binge-like eating episodes induced by frustration stress in female rats (Micioni Di Bonaventura et al., 2014), we further investigated HPA axis activity and regulation of CRF expression in the brain in BER and BEP rats.

Foot shock stress led to a significant increase in plasma corticosterone levels in BER rats that was accompanied by strong stress-induced activation of the expression of CRF mRNA in the PVN. Stress-induced activation of CRF expression in the PVN in BER rats may contribute to decreased chow and energy intake after exposure to stress in this phenotype. Intracerebroventricular (icv) or intra-PVN administration of CRF decreased food intake and increased energy expenditure, which resulted in a sustained decrease in body weight gain in rodents (Buwalda, de Boer, Van Kalkeren, & Koolhaas, 1997; Hotta et al., 1991; Krahn, Gosnell, Levine, & Morley, 1988). Conversely, the anorectic effect of stress has been prevented by a blockade of the CRF receptors (Krahn, Gosnell, Grace, & Levine, 1986; Smagin, Howell, Redmann, Ryan, & Harris, 1999). Interestingly, BER rats showed a stress-induced decrease in chow but not in sucrose intake. These differential effects of stress on regular versus palatable food are in agreement with earlier results showing the anorectic effects of restrain stress on chow but not on sucrose intake in male rats (Martin & Timofeeva, 2010).

In contrast to the BER rats, the plasma corticosterone levels and CRF mRNA expression in the PVN were not affected by stress in the BEP rats. Access to palatable food such as lard and/or sucrose can effectively blunt the corticosterone response to stress in rodents (Foster et al., 2009; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2010). Sucrose intake also decreased the expression of CRF in the PVN induced by adrenalectomy in rats (Laugero, Bell, Bhatnagar, Soriano, & Dallman, 2001). However, although the BEP rats consumed significantly higher amounts of sucrose than the BER rats, the access sessions to sucrose were similar in terms of duration and frequency for both phenotypes. It has been demonstrated that limited access to a small fixed amount of sucrose is sufficient to attenuate HPA axis responses to stress (Ulrich-Lai et al., 2007). Therefore, the blunted activation of the HPA axis to stress in BEP rats may not be entirely explained by different sucrose intake but should also implicate differential sensitivity of the central CRF system to sucrose intake. In a stress-sensitive mice model deficient in the CRF type 2 receptor (CRF_{2R}), access to a palatable high-fat diet during chronic variable stress resulted in a decreased corticosterone response to restraint stress compared to wild type mice under similar experimental conditions (Teegarden & Bale, 2008). This result supports the hypothesis that individual sensitivity to stress may modulate the effects of palatable food on HPA axis activity. As the BER and BEP rats were selected by their low or high sucrose intake after stress, the differential sensitivity to stress may contribute to the inhibited HPA axis response to stress in the BEP rats. Interestingly, CRF_{2R}-deficient mice subjected to chronic variable stress consumed much higher fat diet compared to their wild type counterparts when the mice had limited access to this palatable diet. Whether the decrease in the stress-induced expression of CRF in the PVN in BEP rats is related to reduced signaling via the CRF_{2R}-related pathways remains to be delineated. In the brain, CRF_{2R} is expressed in the ventromedial hypothalamic nucleus (VMH) and the lateral septum (LS) (Martin & Timofeeva, 2010; Poulin, Lenglos, Mitra, & Timofeeva, 2012). Both areas receive direct projections from the PVN (Pittman, Blume, & Renaud, 1981; Ter Horst & Luiten, 1987) and mediate anorectic effects of CRF agonists (Bakshi, Newman, Smith-Roe, Jochman, & Kalin, 2007; Chao, Digruccio, Chen, & Li, 2012; Chen, Hover, Lindberg, & Li, 2012; Chen, Vaughan, Donaldson, Vale, & Li, 2010). Overeating of sucrose in male rats developed by repeated episodes of food restriction and intermittent access to sucrose was accompanied by a decrease in CRF_{2R} mRNA expression in the VMH and the LS (Martin & Timofeeva, 2010).

In contrast to the blunted stress-induced increase in CRF expression in the PVN, the BEP rats demonstrated high CRF mRNA expression in the oval and anteroventral BNST nuclei in response to stress. The BNST is an important region in the brain for the expression of motivated behavior and is considered a link between stress and reward systems (Flavin & Winder, 2013). It has been previously shown that CRF mRNA expression was increased in the BNST after foot shock stress, restraint stress, or pharmacological stressors (Funk, Li, & Le, 2006). CRF signaling in the BNST has also been associated with the stress-induced reinstatement of drug- and alcohol-seeking (Buffalari & See, 2011; Erb & Stewart, 1999; Silberman, Matthews, & Winder, 2013; Wang, Fang, Liu, & Lu, 2006). Intra-BNST injections of CRF induced reinstatement of drug-seeking (Erb & Stewart, 1999), whereas administration of CRF receptor antagonists inhibited stress-induced relapse (Erb & Stewart, 1999; Wang et al., 2006). The origin of CRF involved in the intra-BNST CRF-induced reinstatement of drug-seeking is not clearly determined, but it seems that CRF neurons projecting from the CeA as well as the local BNST population of CRF neurons are important in this mechanism (Erb, Salmaso, Rodaros, & Stewart, 2001; Kash, Nobis, Matthews, & Winder, 2008; Nobis, Kash, Silberman, & Winder, 2011; Silberman et al., 2013). In a model of binge-like eating induced by frustration stress in repeatedly food-restricted female rats, bingeing rats displayed strong stress-induced neuronal activation of the BNST (Micioni Di Bonaventura et al., 2014). Systemic administration of a CRF type 1 receptor (CRF_{1R}) antagonist and icv or intra-BNST injections of a non-selective CRF receptor antagonist completely reversed stress-induced binge-like eating in this model (Micioni Di Bonaventura et al., 2014) suggesting that CRF signaling in the BNST may be

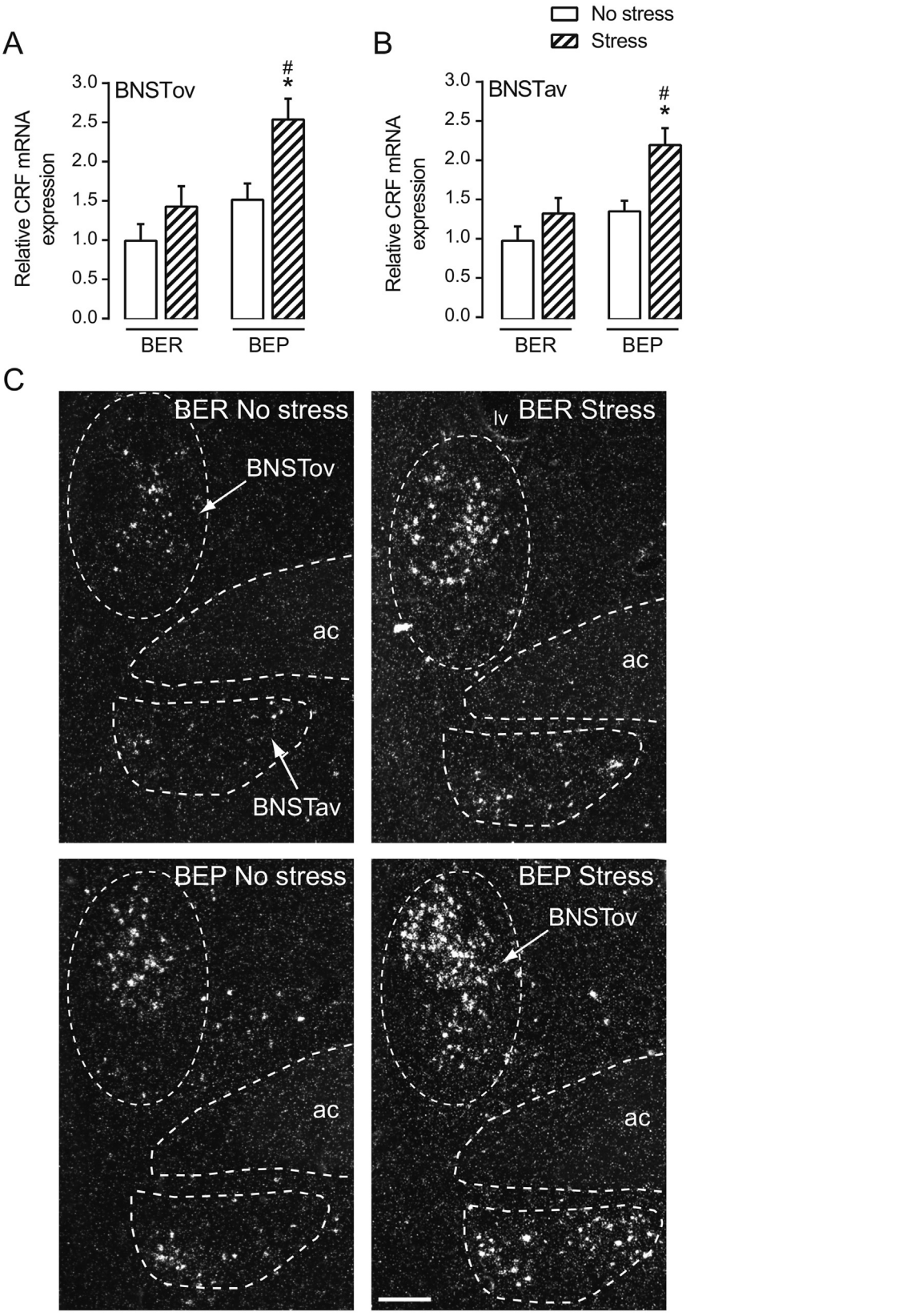

Fig. 4. Relative levels of CRF mRNA expression in the oval (BNSTov, A) and anteroventral (BNSTav, B) nuclei of the bed nucleus of the stria terminalis in BEP and BER rats under non-stressful conditions and after foot-shock stress. (C) Darkfield micrographs illustrating the positive hybridization signal for CRF mRNA in the BNSTov and the BNSTav under non-stressful conditions and following exposure to foot-shock stress in BEP and BER rats. *Significantly ($p < 0.05$) different from the non-stressful condition within the same phenotype; #Significantly ($p < 0.05$) different from BER rats within the same stressful condition. n = 6 rats/group. ac – anterior commissure; BEP, binge-like eating prone rats; BER, binge-like eating resistant rats; lv – lateral ventricle. Scale bar, 200 μm.

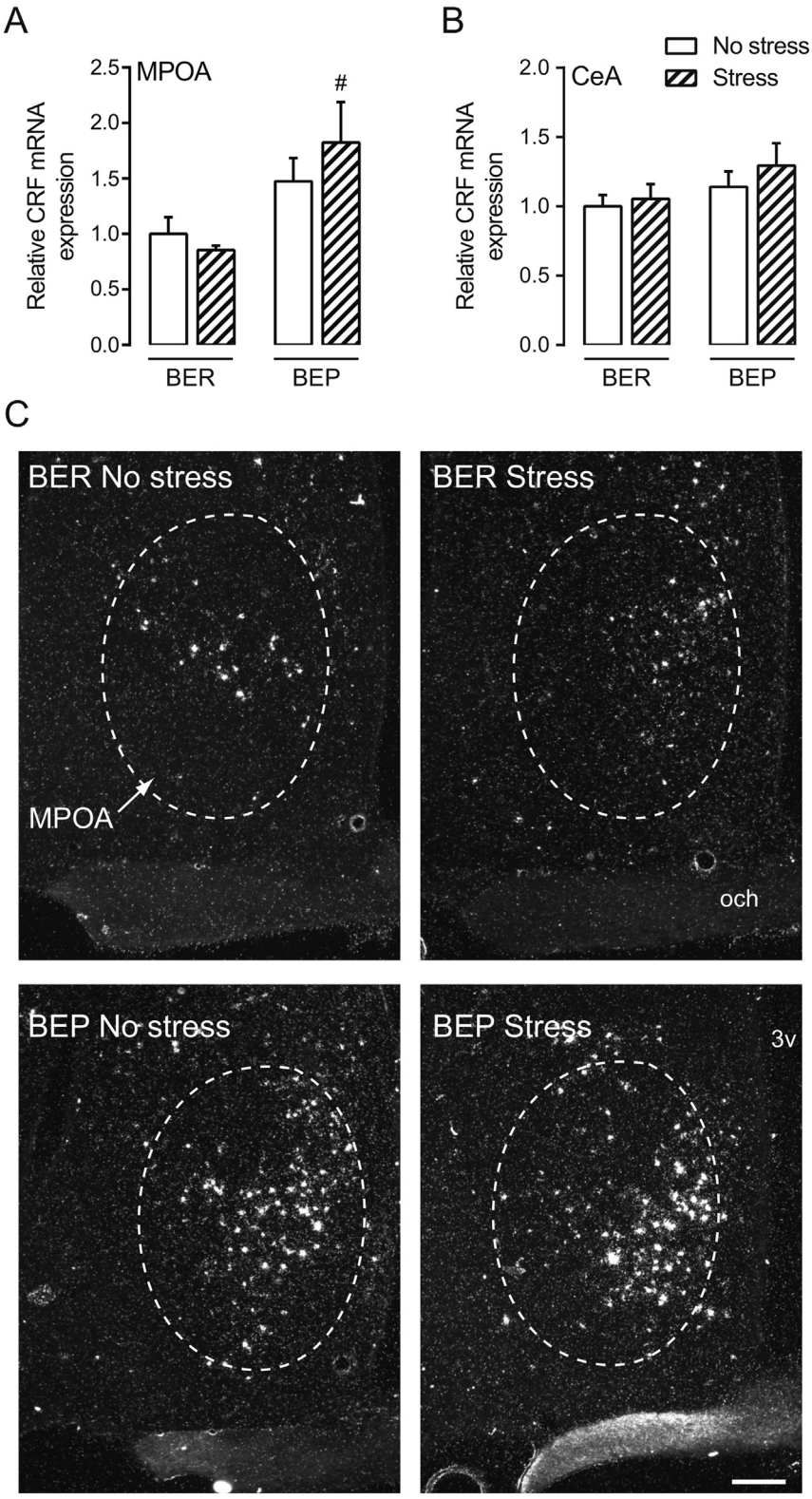

Fig. 5. Relative levels of CRF mRNA expression in the medial preoptic area (MPOA, A) and central amygdala (CeA, B) in BEP and BER rats under non-stressful conditions and after exposure to foot-shock stress. (C) Darkfield micrographs illustrating positive hybridization signal for CRF mRNA in the MPOA under non-stressful conditions and following exposure to foot-shock stress in BEP and BER rats. #Significantly ($p < 0.05$) different from BER rats within the same stressful condition. n = 6 rats/group. 3v – third ventricle; BEP, binge-like eating prone rats; BER, binge-like eating resistant rats; och – optic chiasm. Scale bar, 200 μm.

involved in stress-induced palatable food intake. In our model, the increased CRF expression in the BNST of BEP rats after stress may thus participate in the increased motivation for palatable food and the higher sucrose intake. Reversing the higher sucrose intake of BEP rats after stress with intra-BNST injection of antagonists of CRF receptors would further confirm the involvement of CRF in stress-

induced binge-like eating. The mechanisms of a stress-induced increase in the expression of CRF in the BNST in BEP rats are not yet fully understood. The levels of CRF expression in the BNST were significantly increased in female rats by icv administration of relaxin-3 (Lenglos et al., 2015). Relaxin-3 is a neuropeptide strongly expressed in the pontine nucleus incertus (NI) whose neurons project widely to the forebrain where relaxin-3 binds with high affinity to its native receptor, relaxin family peptide receptor 3 (RXFP3) (Bathgate et al., 2013). A high density of relaxin-3-positive fibers and terminals and high expression of RXFP3 have been detected in the BNST (Ma et al., 2007; Sutton et al., 2004). Direct administration of an RXFP3 antagonist in the BNST significantly decreased self-administration and stress-induced reinstatement of alcohol intake in rats (Ryan et al., 2013). Stress significantly increased expression of relaxin-3 in the NI in BEP rats (Calvez et al., 2016). In addition, the stress-induced increase in sucrose intake in BEP rats was blocked by icv administration of an RXFP3 antagonist (Calvez et al., 2016). Whether the CRF neurons in the BNST are involved in mediating the relaxin-3 effects on stress-induced bingeing on sucrose in BEP rats remains to be investigated.

No difference in CRF mRNA expression was observed between phenotypes and stressful conditions in the CeA in BER and BEP rats. The CeA is an important nucleus in the stress-related circuitry (Herman & Cullinan, 1997; Sawchenko et al., 2000). However, regulation of CRF expression in the CeA seems to be dependent on the stressful conditions and the intensity of stress. Thus, acute restraint stress increased CRF mRNA expression in the CeA in male and female rats (Sterrenburg et al., 2012) while chronic variable mild stress did not affect the levels of CRF mRNA expression in this structure in male and female rats (Sterrenburg et al., 2011). The BNST but not the CeA showed induction of Fos expression in CRF neurons after exposure to stressful and anxiogenic stimuli in high- and low-anxious rats (Butler et al., 2016). An increase in CRF expression in the amygdaloid complex after stress has been shown in another binge-eating model (Pucci et al., 2015); however, binge-like eating in this model was induced by stress exposure and repeated food restriction, which may explain the discrepancy with our model developed without food restriction but based on individual sensitivity to stress.

In summary, this study has shown that rats prone and resistant to sucrose intake after exposure to stress exhibited different patterns of CRF expression following stress sessions that may contribute to the respective higher or lower intake of palatable food in these phenotypes. In the BER rats, stress induced strong activation of the HPA axis. The strong increased expression of anorectic CRF in the PVN of the BER rats may contribute to the lower energy intake of this group in response to stress. In contrast, in the BEP rats, stress did not affect the HPA axis but induced an increase in CRF expression in the BNST that may participate in the increased motivation for sucrose and the high sucrose intake in this group after stress.

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