Hypothalamic CRF1 receptor mechanisms are not sufficient to account for binge-like palatable food consumption in female rats

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Abstract
Objective: The present study evaluated the effect of systemic injection of the CRF1 receptor antagonist R121919, the corticosterone synthesis inhibitor metyrapone and central amygdala (CeA) injections of the nonselective CRF antagonist D-Phe-CRF(12–41) in rats in which binge eating was evoked by stress and cycles of food restriction.

Method: Female rats were subjected or not to repeated cycles of regular chow food restriction/ad libitum feeding during which they were also given limited access (2 h) to palatable food. On the test day, rats were either exposed or not to the sight of the palatable food for 15 min without allowing access, before assessing food consumption.

Results: Systemic injections of R121919, but not of the metyrapone, blocked binge-like eating behavior. Restricted and stressed rats showed up-regulation of crh1 receptor mRNA signal in the bed nucleus of the stria terminalis and CeA but not in basolateral amygdala (BLA) or in the para-ventricular nucleus. Injection D-Phe-CRF(12–41) in CeA but not in the BLA-blocked binge-like eating behavior.

Discussion: These findings demonstrate that extra-hypothalamic CRF1 receptors, rather than those involved in endocrine functions, are involved in binge eating and the crucial role of CRF receptors in CeA. CRF1 receptor antagonism may represent a novel pharmacological treatment for binge-related eating disorders.

KEYWORDS
binge eating, BNST, CeA, CRF1 receptor antagonist, HPA axis, stress

1 | INTRODUCTION

Binge-eating episodes represent a core behavioral feature of binge-eating disorder (BED), bulimia nervosa, and binge/purge subtype anorexia nervosa (American Psychiatric Association, 2013). Episodes of binge-eating in humans are characterized by compulsive, nonhomeostatic consumption of an unusually large quantity of highly palatable food in a short period of time. Binge eating is also an additional feature of obese individuals, significantly contributing to their high caloric intake and finally being overweight (Hudson, Hiripi, Pope, & Kessler, 2007; Swanson, Crow, Le Grange, Swendsen, & Merikangas, 2011). Even in the absence of hunger, some subjects exhibit episodes of rapid eating beyond satiation accompanied by an uncontrollable urge to obtain and consume food. This condition resembles behavioral phenomena described in drug dependence and similarities between binge eating and addiction have been proposed (Avena, 2011; Avena, Bocarsly, Hoebel, & Gold, 2011; Corwin & Grigson, 2009; Corwin, 2010; Corwin & Grigson, 2009; Cottone et al., 2009; D’Addario et al., 2014; Johnson & Kenny, 2010; Volkow, Wang, Fowler, Tomasi, & Baler, 2012).

A large body of evidence suggests that dieting, stress, and negative affect represent possible triggers of binge eating in patients suffering...
from BED or bulimia nervosa (Freeman & Gil, 2004). Indeed, dieting periods are a common finding in the history of people with binge-eating disorder, although hunger per se appears to be insufficient to induce binge eating in the absence of stress and negative affective state (Polivy, Zeitlin, Herman, & Beal, 1994). The important role of stress in the etiology of binge eating is emphasized by the finding that obese individuals with BED exhibit activation of the hypothalamic–pituitary–adrenal (HPA) axis, and their cortisol levels are higher in comparison to those of obese individuals without BED (Glück, Geliebter, Hung, & Yahav, 2004). Moreover, higher blood cortisol levels in response to stress predict greater intake of sweets (Epel, Lapidus, Spagnol, & Appolinario, 2007). Thus the increase of corticosterone levels represent a hormonal marker of binge eating induced by cyclic food restrictions and stress (Artiga et al., 2007; Cifani et al., 2010; Cifani, Polidori, Melotto, Ciccocioppo, & Massi, 2009) and palatable food intake has been shown to blunt activation of the HPA axis (Christiansen, Dekloet, Ulrich-Lai, & Herman, 2011; Kinzig, Hargrave, & Honors, 2008) suggesting that it may represent a kind of self-medication in conditions of stress. The CRF system initiates the neuroendocrine response to stress via the HPA axis, and coordinates several behaviors via actions on extra hypothalamic neural substrates. At extra hypothalamic sites, including the central amygdala (CeA) and the basolateral amygdala (BLA), CRF coordinates the affective reactions to stress (Gray, 1993; Wang et al., 2011). CRF1 is the most abundantly expressed CRF receptor in the brain and high density has been reported in the hypothalamus, in the ventral tegmental area, amygdala, and various cortical structures. Expression of CRF2 receptors is more restricted and is primarily detected in the hypothalamus, dorsal raphe, and lateral septum (Bale & Vale, 2004; Fekete & Zorrilla, 2007; Schank, Ryabinin, Giardino, Ciccocioppo, & Heilig, 2012).

It is well known the involvement of CRF system on CRF-induced anorexia (Cabanac & Richard, 1995; Ciccocioppo et al., 2002; Ciccocioppo, Martin-Fardon, Weiss, & Massi, 2001; Heinrichs et al., 1996; Heinrichs, Lì, & Iyengar, 2001; Heinrichs & Richard, 1999; Richard, Lin, & Timofeeva, 2002) and stress-induced anorexia (Epstein et al., 2016; Harris, 2017; Stengel & Tache, 2014), or hypophagia for palatable food (Cottone et al., 2009; Fedeli et al., 2009; Iemolo et al., 2013; Zorrilla et al., 2004).

In particular CRF1 receptor antagonists have been reported to reduce stress-induced palatable food-seeking (Ghitza, Gray, Epstein, Rice, & Shaham, 2006) and to reduce withdrawal symptoms in conditions of intermittent access to palatable food (Cottone et al., 2009) in rats, and also to reduce palatable food craving and eating in restrained eaters (Epstein et al., 2016).

We recently reported that systemic injections of the CRF1 receptor antagonist R121919 and bed nucleus of the stria terminalis (BNST) injections of the CRF receptor antagonist D-Phe-CRF12–41 decreased frustration stress-induced binge eating in rats with a history of food restriction (Micioni Di Bonaventura et al., 2014).

These data suggest the possibility that CRF-induced modulation of binge eating is not only linked to peripheral corticosteroid hormonal release subsequent to HPA activation but also with the involvement of extra hypothalamic mechanisms.

To explore this possibility, we used an animal model in female rats, in which binge-like eating was elicited by a history of cyclic food restriction and an acute frustration stress (exposure to a familiar palatable food, without being allowed access to it). This stress procedure has face validity as a model of the putative contributions of dieting and negative-valence states, to binge eating in humans (Association, 2013; Mathes, Brownley, Mo, & Bullik, 2009; Sanislow et al., 2010; Treasure, Claudino, & Zucker, 2010; Vannucci et al., 2015). Considering the high prevalence of binge eating in women (Hudson et al., 2007; Swanson et al., 2011), we used female rats, in which binge-like eating behavior varies across the estrus cycle (Alboni et al., 2017; Micioni Di Bonaventura et al., 2017), similarly to women (Culbert, Racine, & Klump, 2016; Edler, Lipson, & Keel, 2007; Klump et al., 2013; Schoofs, Chen, Braunig, Stamn, & Kruger, 2011) and this result increases the validity of the model that can be used in translational studies of the mechanism of binge-eating behavior.

Here, using this rat model, we studied the role of CRF system in stress versus nonstress conditions, in food restricted versus nonfood restricted conditions and both stress plus restriction that induce binge eating. We investigate the effect of exogenous corticosterone administration and metyrapone-induced corticosterone synthesis inhibition and the effect of systemic injection of the selective CRF1 antagonist R121919. Finally, guided by the analysis of crhr1 receptor (crhr1) mRNA transcripts in the brain, using the nonselective CRF1/2 peptidergic antagonist D-Phe-CRF12–41, we evaluated the effect of blockade of CRF receptors in the CeA and the BLA on binge eating.

2 METHOD

2.1 Experiment 1. Effect of the CRF1 receptor antagonist R121919 or metyrapone on binge eating

We determined the effect of systemic injection of R121919 on frustration stress-induced binge-like eating to compare its effect with that of metyrapone, tested in the same rats.

We used 108 female Sprague-Dawley rats that were divided into 12 groups (n = 9 per group) in a 2 (history of intermittent food restriction: no, yes) × 2 (stress during testing: no, yes) × 3 (R121919 dose: 0, 10, 20 mg/kg) factorial design. We injected the rats with vehicle or R121919, 60 min before of the 2 h palatable food access.

These rats were exposed (or not exposed) for 24 days (d) to three 8-d cycles of food restriction (66% of chow intake on d 1–4 and free feeding on d 5–8 of each cycle) during which they were given access to palatable food (prepared by mixing of Nutella (Ferrero, Italy) chocolate-hazelnut cream, chow, and water as described in Supporting Information Methods) for 2 h during the light cycle on d 5–6 and 13–14 of the first two cycles (total of four exposures). On the binge intake test day, we assessed palatable food intake for 2 h immediately after exposure or not to frustration stress (Figure 1a). This stress procedure was adopted to generate a mild stressful condition triggered by the view of palatable food and causes a significant increase in serum corticosterone.
levels (Cifani et al., 2010; Cifani, Di Bonaventura, Ciccocioppo, & Massi, 2013; Micioni Di Bonaventura, Vitale, Massi, & Cifani, 2012).

In previous studies we observed that in the estrous phase of the ovarian cycle, female rats do not exhibit binge eating using our model (Alboni et al., 2017; Micioni Di Bonaventura et al., 2017) while in all other three phases, the rats exhibit binge eating without significant differences in intensity. Therefore, to control for the estrous cycle, immediately after the test on d 25, vaginal smears were collected and analyzed under microscope by an experimenter blind to treatment conditions. Data from rats in the estrous phase were not included in the statistical analysis.

As reported in our previous studies (Cifani et al., 2009; Micioni Di Bonaventura et al., 2013; Piccoli et al., 2016), after 1 d off at the end of the first test, the same animals received an additional 8-d cycle: the nonrestricted groups had 8 d of chow ad libitum, whereas the restricted groups had 4 d of chow restricted to 66% of the normal intake followed by 4 d of chow ad libitum. In this additional cycle, all groups did not have access to palatable food. The following day, the stressed groups were exposed to stress while the nonstressed groups were not. On the test day, 1 h before access to palatable food, rats were treated i.p. with Metyrapone (0, 50, 100 mg/kg).

2.2 | Experiment 2. Effect of corticosterone on rats with a history of repeated cycles of food restriction/ad libitum feeding

To assess whether exogenous corticosterone, injected in rats submitted to three cycles of food restriction but not exposed to stress evokes binge eating, 63 female rats were used.

They were divided into two groups, matched for body weight and daily food intake: (a) nonrestricted and not exposed to stress group (n = 27); (b) restricted and not exposed to stress group (n = 27). A third group (restricted and stressed rats, n = 9), treated only with vehicle, was added to compare the binge-eating response to that of the other groups treated with corticosterone.

Rats were submitted to three consecutive 8-d cycles followed by the final test on d 25 as described in Experiment 1.

On the test day (d 25), nonstressed animals were divided into subgroups of nine rats and treated i.p. with corticosterone (2.5 or 5 mg/kg), or its vehicle, 30 min before access to palatable food.

After 1 d off at the end of the first test, the same animals received an additional 8-d cycle as described above. On the test day, animals were divided into three subgroups of nine rats and treated i.p. with corticosterone (10 or 15 mg/kg), or its vehicle, 30 min before access to palatable food.

2.3 | Experiment 3. In situ hybridization analysis of crr1 mRNA transcripts in the BNST, CeA, BLA, and paraventricular nucleus

For the in situ hybridization experiment, 36 rats were divided into four groups (n = 9/group) in a 2 (history of intermittent food restriction: no, yes) × 2 (stress during testing: no, yes) factorial design. These rats were exposed (or not exposed) for 24 d to three 8-d cycles of food restriction. On the binge intake test day, they were exposed or not to frustration stress as described above. On d 25, rats were sacrificed by decapitation.

2.4 | Experiment 4. Effect of CeA and BLA injection of D-phe-CRF(12-41) on binge eating

We determined the effect of CeA and BLA injections of the CRF receptor antagonist D-Phe-CRF(12-41) on binge eating.

For CeA microinjection we used 32 rats that were divided into four groups (n = 8/group). Two groups were not exposed to cycles of food restriction and frustration stress and were tested for the effect of D-Phe-CRF(12-41) (0, 300 ng/side) on palatable food intake during the 2 h test on d 25. Two other groups were exposed to three cycles of food restriction and frustration stress and were tested for the effect of D-Phe-CRF(12-41) (0, 300 ng/side) on stress-induced expression of binge eating. We injected D-Phe-CRF(12-41) 30 min before the 2 h palatable food access (15 min before the beginning of the frustration stress for the stressed groups).

For BLA microinjection we used another 32 rats divided into four groups (n = 8/group); the experimental procedure was the same described above for CeA microinjection experiment.

Additional methodological details are contained in Supporting Information, Methods.

3 | RESULTS

3.1 | Experiment 1

As in our previous studies (Cifani et al., 2010; Cifani et al., 2009; Micioni Di Bonaventura, Cifani, et al., 2012; Micioni Di Bonaventura, Vitale, et al., 2012; Pucci et al., 2016), body weight of rats was reduced during the 4 d of food restriction, but immediately afterwards the animals increased their food intake and rapidly recovered their body weight to levels of controls by the end of each cycle. On the test day body weight of animals, as well as their food intake in the previous 24 h, were not significantly different among the groups (data not shown).

Thirty-two rats (of the 108) were excluded from the analysis because of estrous on the test day.

At 15 min, a three-way ANOVA, which included the between-subjects factors of history of intermittent food restriction (no, yes), stress during testing (no, yes), and R121919 dose (0, 10, 20 mg/kg), showed a significant interaction among the three factors [F(2,64) = 3.5, p < .05]. Post hoc test showed an increase of palatable food consumption during the first 15 min of the feeding test on Day 25 (p < .01) (Figure 1b, left panel), only in vehicle rats with a history of intermittent food restriction and frustration stress (binge-eating group). Post hoc test revealed also that systemic injections of R121919 in restricted and stressed rats decreased palatable food consumption during the first 15 min of the feeding test (p < .01) (Figure 1b, left panel). In contrast, R121919 injections had no effect on palatable food intake in the other three groups (p > .05).
ANOVA of the 2 h cumulative palatable food showed a three-way interaction (food restriction, stress, and R121919 dose) $F(2,64) = 3.3, p < .05$. As shown in Figure 1b (right panel), the post hoc test revealed that 2 h cumulative palatable food intake was significantly increased in the vehicle restricted and stressed group in comparison to the other vehicle groups ($p < .01$). Moreover, the post hoc test showed that the
treatment with R121919 (20 mg/kg) significantly reduced palatable food intake in restricted and stressed group \(p < .01\) (Figure 1b, right panel).

In the second test with metyrapone treatment, 31 female rats (of the 108 used in the experiment) were excluded from the analysis because of estrous on the test day.

At 15 min, overall ANOVA which included the between-subjects factors of history of intermittent food restriction (no, yes), stress during testing (no, yes), and corticosterone dose (0, 50, 100 mg/kg), showed a significant interaction between food restriction and stress \(F(1,66) = 33.7, p < .01\). As shown in Figure 1c (left panel), 15 min palatable food consumption in vehicle rats with a history of food restriction, and exposed to 15 min frustration stress was significantly higher in comparison to the other vehicle groups. Systemic injections of metyrapone did not reduce palatable food intake in any group of rats.

ANOVA of the 2 h cumulative palatable food showed a significant interaction between food restriction and stress \(F(1,66) = 14.8, p < .01\). As shown in Figure 1c (right panel), 2 h cumulative palatable food intake was significantly increased only in the vehicle restricted and stressed group in comparison to the other vehicle groups. The treatment with metyrapone did not affect feeding in any group.

### 3.2 Experiment 2

Twenty rats (of the 88 used in the experiment) were excluded from the analysis because of estrous on the test day.

The statistical analysis of the first 15 min palatable food intake, which included the between-subjects factors of history of intermittent food restriction (no, yes), and corticosterone dose (0, 2.5, 5.0 mg/kg), did not show a significant interaction between two factors \(F(6,99) = 0.5, p > .05\) (Figure 2a, left panel).

ANOVA of the 2 h cumulative palatable food did not show a two-way interaction (food restriction, and corticosterone dose) \(F(2,33) = 0.13, p > .05\) (Figure 2a, right panel).

After an additional food restriction/ad libitum feeding cycle, higher doses of corticosterone (10, 20 mg/kg) also did not produce significant interactions between the two factors during the first 15 min \(F(6,105) = 1.9, p > .05\) (Figure 2b, left panel) or cumulatively through the 2 h session \(F(2,35) = 0.32, p > .05\) (Figure 2b, right panel).

Overall ANOVA comparing the vehicle group (nonrestricted and nonstressed; restricted and nonstressed; restricted and stressed) showed significant group interaction in the first experiment \(15\) min: \(F(2,16) = 21.2, p < .01\) [2 h: \(F(2,16) = 4.2, p < .05\)] and in the second experiment \(15\) min: \(F(2,15) = 15.3, p < .01\) [2 h: \(F(2,16) = 13.1, p < .01\)]. Post hoc test showed that the palatable food intake of restricted and stressed rats (binge-eating group) treated with vehicle was significantly higher in comparison to the other two vehicle groups both in the first test \(15\) min \(p < .05\) and cumulative 2 h \(p < .01\) and in the second test \(15\) min \(p < .05\) and cumulative 2 h \(p < .01\).

### 3.3 Experiment 3

For the in situ hybridization 12 rats were not used because of estrous on the test day.

For crhr1 mRNA levels, overall two-way ANOVA with history of intermittent food restriction (no, yes), and stress during testing (no, yes) as between factors, showed a significant interaction in dorsal BNST \(F(1,20) = 3.2, p < .05\), but not in ventral BNST \(F(1,20) = 2.9, p > .05\). Post hoc test showed that in dorsal BNST crhr1 mRNA levels were significantly higher \(p < .05\) in restricted and stressed rats compared to the nonrestricted and nonstressed rats (Figure 3a).

In the CeA overall ANOVA revealed also a significant interaction between the two factors of history of intermittent food restriction and stress \(F(1,20) = 3.9, p < .05\). As shown in Figure 3c, post hoc test revealed a higher level \(p < .01\) of crhr1 mRNA in restricted and stressed rats compared to the nonrestricted and nonstressed rats.

Two-way ANOVA showed that crhr1 mRNA levels in BLA were significantly affected by frustration stress \(F(1,20) = 14.5, p < .01\) but not by restriction \(F(1,20) = 0.6, p > .05\). No interaction between these two factors was detected \(F(1,20) = 0.002, p > .05\) (Figure 3d).

Two-way ANOVA showed that crhr1 mRNA levels in paraventricular nucleus (PVN) were not significantly affected by frustration stress \(F(1,20) = 1.4, p > .05\) nor by restriction \(F(1,20) = 0.8, p > .05\). No significant interaction between these two factors was detected \(F(1,20) = 0.8, p > .05\) (Figure 3e).

### 3.4 Experiment 4

Fourteen female rats (of the 64 used in the experiment) were excluded from the statistical analysis because of estrous on the test day.

After histological evaluation for correct cannula placement nine (three were already excluded because in the estrous phase) of the 64 rats were excluded from the analysis because of placements outside the CeA or BLA. Representative brains with correct cannula placements in CeA and in BLA are shown in Supporting Information, Figure S1.

The statistical analysis for microinjection in CeA, which included the between-subjects factors of group condition (nonrestricted and nonstressed and restricted and stressed groups) and D-Phe-CRF\(_{12-41}\) dose (0, 300 ng/side), showed a significant interaction between the two factors \(F(1,19) = 7.5, p < .05\), at 15 min.

ANOVA of the 2 h cumulative palatable food intake showed a significant interaction between the two factors \(F(1,19) = 4.9, p < .05\).

As shown in Figure 4, post hoc test revealed a significant increase in palatable food consumption in the restricted and stressed groups during the first 15 min \(p < .01\) and at the end of the test (2 h cumulative food intake) \(p < .05\). Moreover, the treatment with D-Phe-CRF\(_{12-41}\) (300 ng/side) significantly reduced palatable food intake only in restricted and stressed group at 15 min \(p < .01\).

The statistical analysis for microinjection in BLA did not show a significant interaction between the two factors (group condition, and
D-Phe dose) at 15 min \( F(1,17) = 0.2, p > .05 \) and 2 h \( F(1,17) = 0.02, p > .05 \) (data not shown).

4 | DISCUSSION

In previous work, we found that systemic injections of the CRF1 receptor antagonist R121919 or intra-BNST administration of the CRF receptor antagonist D-Phe-CRF(12-41) selectively decreased frustration stress-induced binge eating in female rats with a history of food restriction. These compounds did not affect palatable food intake in control rats (Micioni Di Bonaventura et al., 2014).

It is known that binge eating is also associated with stimulation of HPA axis activity and elevation of peripheral corticosteroids (Artiga et al., 2007; Cifani et al., 2010; Cifani et al., 2009). Activation of CRF1 receptors in the PVN is a primary mechanism responsible for HPA axis...
activation by stress, whereas at extrahypothalamic sites (i.e., BNST, CeA, BLA, etc) CRF1 modulates affective and emotional reactions. We sought therefore, importantly, to determine whether the effect of CRF1 receptor antagonist on binge eating is linked to their ability to blunt the activity of HPA axis system.

To examine this possibility, we tested the effect of metyrapone, a corticosterone synthesis inhibitor, to inhibit binge eating, but it did not reduce palatable food intake in any experimental group. To confirm this finding, after three cycles of food restriction and ad libitum feeding, we replaced the frustration stress with multiple concentrations of

**FIGURE 3** Restricted and stressed rats showed up-regulation of crh1 receptor mRNA signal in the BNST and CeA but not in BLA or in PVN. (a) crhr1 mRNA levels in dorsal BNST, (b) ventral BNST, (c) CeA, (d) BLA, and (e) PVN. *p < .05; **p < .01, different from the nonrestricted and nonstressed group. n = 6 per group.
corticosterone, but it failed to elicit binge eating. Taken together, these findings do not support a critical role for HPA axis in the expression of this food-related maladaptive behavior in female rats. Then we focused our attention to the regulation of CRF1 receptors at extrahypothalamic sites: we determined expression of the crhr1 mRNA in restricted and stressed rats showing binge-eating behavior or their controls. Data revealed a significant up-regulation of crhr1 mRNA levels in the dorsal portion of the BNST and in the CeA only in restricted and stressed rats, whereas no changes were detected in the BLA and in the PVN in any groups of rats.

Lack of changes in the PVN provides a further confirmation for a limited role of hypothalamic CRF1 receptor mediated mechanisms in binge-eating expression, whereas it is extremely interesting to observe over expression of the of crhr1 transcripts both in the BNST and CeA. Considering these in situ hybridization data, nonselective CRF receptor antagonist D-Phe-CRF(12–41) was administered into the CeA and the BLA. Blockade of CRF receptors in the CeA decreased binge eating in restricted and stressed rats, whereas no effect was detected in the BLA. In this work we focused on the amygdala, because we already reported the important role of BNST in the expression of binge eating (Micioni Di Bonaventura et al., 2014); we found an enhancement of c-fos levels in binge female rats in this brain region and the D-Phe-CRF(12–41) administration completely blocked this behavior selectively in restricted and stressed rats.

The amygdala and BNST are brain regions anatomically and functionally connected (de Olmos & Heimer, 1999) and the amygdala is known to have CRF-containing projection to the BNST (Erb, Salmaso, Rodaros, & Stewart, 2001) and in particular CeA has emerged as an important area in the regulation of excessive consumption of palatable food (Blasio et al., 2013; de Olmos & Heimer, 1999; Iemolo et al., 2013; Micioni Di Bonaventura et al., 2017). The observation that blockade of CRF receptors in these brain areas completely reversed stress-induced binge eating in our model indicates a critical role of extrahypothalamic CRF systems. Moreover, both CRF antagonists tested, centrally and peripherally injected, selectively blocked frustration stress-induced binge eating after a history of food restriction, without affecting only stressed or only restricted rats.

In this regard, it is also important to consider that the effect of CRF1 receptor antagonism on binge eating, appears to be unrelated to the regulation of satiety mechanism or caloric intake, as we detected an effect only when consumption was triggered by stress and associated with a loss of control. This finding is in agreement with Parylak et al. (2012) report, showing that systemic injections of R121919 has no effect on binge-like eating in female rats not exposed to stress in a different binge-eating model (Parylak, Cottone, Sabino, Rice, & Zorrilla, 2012). Taking into account this selective effect, it is also possible to exclude with these compounds and these doses malaise and taste aversion, that CRF antagonism can produce (Heinrichs et al., 1998) and influence satiety or feeding behavior per se.

This work extends to our previous results on the role of CRF receptors and supports further investigation to understand how CRF antagonists block feeding consumption in different animal conditions, such as intermittent access to chocolate flavored, high-sucrose diet (Cottone et al., 2009), or reduce stress-induced reinstatement of palatable food seeking (Ghitza et al., 2006).

Moreover, in humans, promising anti-craving properties were exhibited in the presence of the CRF1 antagonist, pexacerfont,
especially in the presence of palatable food in restrained eaters (Gold, Frost-Pineda, & Jacobs, 2003). Unfortunately, this clinical study was interrupted because of only an administrative interpretation of US federal law and not for the course of study.

In conclusion, the results of the present study do not support a direct role of the HPA axis and corticosterone in the expression of binge eating in female rats. Eating disorders share important commonalities with substance abuse (Avena et al., 2011; Shepard, Barron, & Myers, 2000), for example, high circulating levels of glucocorticoids can sensitize the CRF systems in extrahypothalamic sites such as the CeA and norepinephrine systems in the BLA, that are known to be involved in behavioral responses to stressors (Imaki, Nahan, Rivier, Sawchenko, & Vale, 1991; Shepard et al., 2000). Thus, it may be hypothesized that activation of the HPA axis may lead to subsequent activation of extrahypothalamic brain stress systems, as described for drug addiction (Koob & Le Moal, 2005; Koob & Kreek, 2007; Kreek & Koob, 1998), and that CRF1 receptors may be involved in these effects.

5 | CONCLUSION

The findings of the present study provide clear evidence that CRF is involved in the binge-eating response to stress and food restrictions in female rats, and the crucial role of CRF receptors in BNST and CeA; this effect may be related to extrahypothalamic effects of CRF and CRF1 receptor antagonists may represent interesting tools for the pharmacotherapy of binge-eating related disorders.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

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