Published in final edited form as:

Int J Eat Disord. 2017 June ; 50(6): 624–635. doi:10.1002/eat.22687.

Estrogenic suppression of binge-like eating elicited by cyclic food restriction and frustrative-nonreward stress in female rats

Maria Vittoria Micioni Di Bonaventura¹, Thomas A. Lutz², Adele Romano³, Mariangela Pucci⁴, Nori Geary⁵, Lori Asarian^{2,*}, and Carlo Cifani^{1,*}

¹School of Pharmacy, Pharmacology Unit, University of Camerino, 62032 Camerino, Italy

²Institute of Veterinary Physiology and Center for Integrative Human Physiology, University of

Zurich, 8057 Zurich, Switzerland ³Department of Physiology and Pharmacology, Sapienza

University of Rome, 00185 Rome, Italy ⁴Faculty of Bioscience and Technology for Food,

Agriculture and Environment, University of Teramo, 64100 Teramo, Italy ⁵Department of

Psychiatry (Retired), Weill Medical College of Cornell University, New York, NY 10065, USA

Abstract

Objective—Because binge eating and emotional eating vary through the menstrual cycle in human females, we investigated cyclic changes in binge-like eating in female rats and their control by estrogens.

Method—Binge-like eating was elicited by three cycles of 4 days of food restriction and 4 days of free feeding followed by a single frustrative nonreward-stress episode (15 min visual and olfactory exposure to a familiar palatable food) immediately before presentation of the palatable food.

Results—Intact rats showed binge-like eating during the diestrous and proestrous phases of the ovarian cycle, but not during the estrous (peri-ovulatory) phase. Ovariectomized (OVX) rats not treated with estradiol (E2) displayed binge-like eating, whereas E2-treated OVX rats did not. The procedure did not increase signs of anxiety in an open-field test. OVX rats not treated with E2 that were subjected to food restriction and sacrificed immediately after frustrative nonreward had increased numbers of cells expressing phosphorylated extracellular signal-regulated kinases (ERK) in the central nucleus of the amygdala (CeA), paraventricular nucleus of hypothalamus (PVN), and dorsal and ventral bed nucleus of the stria terminalis (BNST) compared with non-restricted or E2-treated rats.

Discussion—These data suggest that this female rat model is appropriate for mechanistic studies of some aspects of menstrual-cycle effects on emotional and binge eating in human females, that anxiety is not a sufficient cause of binge-like eating, and that the PVN, CeA and BNST may contribute to information processing underlying binge-like eating.

Corresponding author: Lori Asarian, Institute of Veterinary Physiology and Center for Integrative Human Physiology, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland, lasarian@vetphys.uzh.ch.

*Share senior authorship

Conflict of Interest: The authors declare no competing financial interests.

Keywords

pERK; stress; binge eating; estradiol; bulimia nervosa; binge-eating disorder; emotional eating; menstrual cycle; ovarian cycle

Binge eating, eating an excessive amount of food in a discrete time with an accompanying sense of lack of control over eating, is the central feature of bulimia nervosa, in which individuals compensate for the increased food intake, e.g., by purging, and binge-eating disorder, in which individuals do not compensate.¹ and, therefore, are frequently obese.¹ Binge eating disproportionately affects females, whose lifetime prevalence of bulimia nervosa (~1.3–1.5% in the USA) and binge-eating disorder (~2.3–3.5%) are ~2-fold those of males.^{2,3} Binge eating also varied through the menstrual cycle. During the luteal phase, when estrogens and progestins are both high, binge eating rates are higher than during the mid- to late-follicular or peri-ovulatory phases, when only estrogens are high. This was shown in several studies of females with bulimia nervosa^{4,5} and in one study of females with binge-eating disorder and bipolar disorder.⁶ Similar menstrual-cycle variations occur in women's tendency for emotional eating, i.e., excessive intake stimulated by negative affect, which is a risk factor for eating disorders.^{4,7,8} In the largest such study to date, Klump et al.^{9,10} quantitatively analyzed the relations among menstrual fluctuations in levels of estradiol (E2, the main estrogen), progesterone (the main progestin), emotional eating scores¹¹ and binge eating. The results supported a model in which estrogens inhibit emotional-eating and progestins oppose this effect of estrogens, i.e., indirectly stimulate emotional eating. Importantly, the results were clearest in women who reported engaging in binge episodes; i.e., whose eating was most dysregulated.

Because the biological factors contributing to the etiology of binge eating are not well understood,^{12–14} animal models are an increasingly important facet of binge-eating research.^{15,16} Binge-like eating can be elicited in rodents simply by intermittent access to palatable food.^{15,17–25} Such limited-access models capture some of the sex differences in human binge eating. For example, female rats were markedly more prone than male rats to display binge-like eating when palatable mix of sugar and fat was offered on an intermittent schedule.²² When fat alone was provided on a similar intermittent schedule, however, female rats ate much less than males during binge-like-eating tests.^{18,21} Furthermore, when ovariectomized (OVX) rats were offered fat intermittently and E2 and progesterone were cyclically injected to mimic the normal estrous (ovarian) cycle, the size of binge-like bouts were reduced tonically, but not cyclically; in addition, apparently in contrast to the human data reviewed above,^{9,10} E2-progesterone and E2 alone had identical effects on binge-like eating.^{24,25}

Other models of binge eating may better capture some aspects of human binge eating than models based on limited access alone. Because the majority of women with eating disorders have a history of dieting and because negative affect often precipitates binge eating,^{13,14} Cifani et al.^{26,27} developed a rat model involving a history of cyclic food restriction and an acute psychological stressor, i.e., a frustrative nonreward procedure consisting of 15 min exposure to the odor and sight of a familiar palatable food, just before offering the food.

Frustrative nonreward and similar aversive experiences are thought to activate the negative-valence system, which includes the neuroendocrine stress system and cortico-limbic neural circuits that produce anxiety, fear, frustration, depression and other negative-emotional states, which in turn contribute to binge eating and other psychopathologies as well as their animal homologues.^{28,29} Importantly, although the frustrative nonreward used in our model increased plasma corticosterone levels, this occurred regardless of food-restriction history, so was not sufficient to account for binge-like eating.^{27,30} The frustrative-nonreward procedure also provides a discrete trigger for binge-like eating, permitting investigation of mechanisms that stimulate binge-like eating in the absence of the eating *per se*, and this has been exploited to investigate the neural mechanisms underlying this form of binge-like eating.³¹ In view of these considerations, we examined whether binge-like eating in this model (1) varies through the ovarian cycle in intact female rats; (2) is influenced by E2 treatment in OVX rats; (3) is associated with increased anxiety or arousal, as measured in an open-field activity test, in intact rats; and (4) is associated with increased neuronal activity, which was assessed immunocytochemically in several brain areas related to eating or to the negative-valence system in E2-treated and untreated OVX rats.

Method

Female Sprague-Dawley rats were housed individually and maintained on standard chow and water. A palatable test food was prepared by mixing of Nutella (Ferrero, Alba, TO, Italy) chocolate-hazelnut cream, chow and water (52%, 33% and 15% weight, respectively). All experiments were carried out in accordance with European and Italian directives.

Experiment 1

Four groups of intact, cycling female rats (n=27 each) were used. One group was subjected to the binge-like eating procedure, done as previously^{26,27,31} with intermittent food restriction and frustrative-nonreward stress prior to 2 h palatable food access, as shown in Figure 1. A second group received only intermittent food restriction, a third group received only frustrative-nonreward stress, and a fourth group received neither food restriction nor frustrative-nonreward stress prior to palatable-food access. Vaginal cytology samples were taken immediately after the binge test and ovarian-cycle phase was assessed using standard criteria³². Four rats were excluded due to the poor quality of the samples. The rats in diestrus 1, diestrus 2 and proestrus were combined into non-estrous groups and compared with rats in estrus. This was done because the only consistent ovarian-cycle effect on spontaneous eating in rats is the decrease during estrus³³, because preliminary observations with the current model indicated that binge-like eating occurred during diestrus and proestrus, but not during estrus.

Experiment 2

The same rats were assigned to the same groups for an additional 8 d food restriction-refeeding or control period and, on the next day, frustrative-nonreward stress (no, yes), after which the rats were placed in automated open-field locomotor-activity boxes (43 × 43 floor area, with a 25 × 25 cm virtual central zone) for 10 min. The open field test was performed immediately after stress without having access to palatable food as in the previous

experiment. Locomotion in the entire open field and in the central zone and entries into the central zone were computed based on interruptions of infrared light beams placed in a 2.6×2.6 orthogonal grid 3.5 cm above the box floor. Increased locomotor activity in the entire field is considered a sign of behavioral arousal, and reduced locomotor activity in the central zone and numbers of entries into the central zone are considered signs of increased emotionality, anxiety or fear in mice and rats^{34–36}. Vaginal cytology samples were collected and analyzed, and rats were assigned to estrous and non-estrous groups for data analysis, as above.

Experiment 3

Rats (n=25) were OVX and, 3 d later, began a previously validated^{33,37} near-physiological E2 regimen (2 µg E2-benzoate in 100 µL sesame oil, SC, once each 4th day) (n=13) or a control regimen (OIL, sesame oil alone) (n=12). After five E2 or OIL cycles, each group was divided into one subgroup that received the intermittent food-restriction and frustrative-nonreward procedure and one group that did not. E2 or OIL treatment was continued, and the binge-eating test was done ~25 h after the final E2 or OIL injection. Neither non-restricted and stressed controls nor restricted and non-stressed controls were included because these groups did not show binge-like eating in *Experiment 1* or in previous studies.^{26,27,31}

Experiment 4

The effects of cyclic food restriction and frustrative-nonreward stress on expression of phosphorylated extracellular signal-regulated kinases (pERK; also known as mitogen-activated protein kinases) were determined in E2– (n=14) and OIL (n=13)-treated OVX rats. Neuronal ERK is phosphorylated in activated neurons in response to increased intracellular calcium and thus indicates activated neurons.^{38,39} The procedure of *Experiment 3* was repeated in a new group of 27 rats, except that on d 25, palatable food was not offered, and rats were prepared for immunocytochemical analysis of pERK expression in the brain areas described below.

In all experiments, data were analyzed with ANOVA and Bonferroni-corrected *post-hoc* t-tests and are reported as mean $\pm$ SEM. Additional methodological details are contained in Supplementary Methods.

Results

Experiment 1

As previously showed,^{26,27,31} restricted rats lost body weight during the 4 d food-restriction periods and regained it during the subsequent 4 d free-feeding periods, such that body weights did not differ significantly across groups on the test day (data not shown). ANOVA of binge-like eating revealed four-way interaction [food restriction $\times$ stress $\times$ ovarian phase $\times$ interval, $F(3,288) = 2.82$, $p < 0.05$]. *Post hoc* tests indicated that during the initial 15 min of the test, restricted and stressed rats tested during diestrus or proestrus (i.e., non-estrous rats) ate more than restricted and stressed rats tested during estrus or than control rats (Figure 2A). In addition, non-estrous rats ate slightly more than estrous rats in the three control

conditions (Figure 2A). Importantly, the relative increase in 15 min palatable-food intake due to restriction and stress in non-estrous rats vs. estrous rats (~100%) was markedly larger than the relative increase in non-estrous rats vs. estrus rats in the three control groups (~25%), indicating a selective interactive effect of restriction/stress and non-estrous status. Rats in all groups ate similar amounts of palatable food during min 15–120 of the test, so that the initial effects were still evident in the 2 h cumulative palatable-food intakes [food restriction x stress x ovarian phase interaction, $F(1,95) = 3.83$, $p < 0.05$] (Figure 2B). Again, the relative increase in 2 h palatable-food intake due to restriction and stress in non-estrous rats vs. estrous rats was larger than the relative increase in non-estrous rats vs. estrous rats in the three control groups (~80% vs. ~20%).

Experiment 2

ANOVA revealed an interactive effect of stress and estrous phase on the distance travelled in the central zone of the open field [$F(1,100) = 18.7$, $p < 0.01$], but no three-way interaction. *Post hoc* tests indicated that stressed non-estrous rats exhibited significantly more locomotor activity in the central zone than non-stressed non-estrous rats regardless of food-restriction history (Figure 3). There was a main effect of stress on the number of entries into the center zone [$F(1,100) = 10.88$, $p < 0.01$], but no interactions. *Post hoc* comparisons revealed that only stressed, restricted, non-estrous rats entered the central zone more often than non-stressed, restricted non-estrous rats. The increase of the locomotion and of the number of entries into the center zone suggests no influence on anxiety-like behavior on binge eating group. Finally, there was an interactive effect of stress and estrous phase distance traveled in the entire open field [$F(1,100) = 7.58$, $p < 0.01$], but no three-way interaction. *Post hoc* tests indicated that food-restricted, stressed non-estrous rats locomoted more than restricted, stressed, estrous rats and more than non-restricted, non-stressed, non-estrous rats, showing an increased of general arousal.

Experiment 3

E2-treated OVX rats gained less weight than OIL-treated OVX rats in the initial 20 d of the experiment (i.e., before the binge like-eating procedure) [main effect of E2/OIL, $F(1,48) = 75.55$, $p < 0.01$] and maintained this difference subsequently, regardless of whether they were food restricted and stressed or not [main effect of E2/OIL, $F(1,48) = 79.30$; $p < 0.01$]. On the test day, neither E2 nor OIL food-restricted, stressed rats differed in weight in comparison with their non-restricted, non-stressed controls [main effect of food restriction and stress, which were grouped as one factor, $F(1,48) = 0.57$; $p > 0.05$] (Figure 4A).

Palatable-food intake on the test day was significantly affected by food restriction-stress and by E2, as indicated by interaction effects between these two factors after both 15 min [$F(1,21) = 6.25$; $p < 0.05$], and 2 h [$F(1,21) = 6.26$; $p < 0.05$]. *Post-hoc* comparisons indicated that 15 min and 2 h palatable-food intakes, first, were less in E2 rats than OIL rats both in the non-restricted, non-stressed rats and the restricted, stressed rats, and, second, were more in restricted, stressed OIL rats than in the other three groups (Figure 4B). Furthermore, the relative increases in 15 min and 2 h palatable-food intakes due to lack of E2 (i.e., OIL vs. E2-treated rats) in food-restricted and stressed rats (~40 and ~30%, respectively) appeared larger than the relative increases in control rats (~25% and ~5%,

respectively), suggesting a selective interactive effect of food restriction-stress and lack of E2.

Experiment 4

The numbers of cells expressing pERK were significantly increased by food restriction-stress and OIL in the CeA, PVN, in dorsal BNST and ventral BNST [interactions of $F(1,19) = 35.8, p < 0.01$; $6.4, p < 0.05$; $5.0, p < 0.05$; and $12.5, p < 0.05$, respectively]. Post-hoc tests indicated that ERK phosphorylation was significantly increased in food-restricted/stressed OIL rats in comparison with the other groups in the CeA, PVN, dorsal BNST, and ventral BNST (Figure 5 and 6). In the ARC there was only a main effect of restriction-stress [$F(1,19) = 4.5, p < 0.01$] (data not shown); in the BLA there were no significant effects (data not shown).

Discussion

Here we report novel studies of the neuroendocrine influence on binge-like eating in a rat model in which binge-like eating was elicited by a history of cyclic food restriction and an acute frustrative-nonreward procedure, in which rats were exposed to a familiar palatable food, without being allowed access to it, immediately before the binge test.^{26,31,40–43} This procedure has face validity as a model of the putative contributions of dieting and negative-valence states, especially psychosocial stress, to emotional and binge eating in humans.^{1,7,8,13,14,28,29}

Binge-like eating and the ovarian cycle in rats and women

Experiment 1 demonstrates for the first time that binge-like eating in intact, cycling female rats varies through the estrous (ovarian) cycle. Rats subjected to the binge procedure that were tested in diestrus or proestrus (i.e., non-estrous rats) displayed ~100% increases in palatable-food intake, i.e., binge-like eating, compared with control rats that were subjected only to cyclic food restriction, only to frustrative nonreward, or to neither. In contrast, binge-like eating in rats tested during estrus was completely suppressed; these rats ate only about as much as estrous rats in the three control groups. Importantly, in all three control conditions, rats tested during estrus ate about ~20% less than rats tested during diestrus or proestrus, which corresponds to the usual cyclic change in food intake in cycling rats.^{33,37} That the decrease in binge-like eating in estrus was relatively much larger than this typical cyclic effect indicates that it is a distinct phenomenon, not simply a reflection of the ovarian rhythm of spontaneous eating.

This cyclic variation in binge-like eating in rats is superficially similar to the regular menstrual cycle-related variations in binge frequency in women with bulimia nervosa,^{4,5} binge-eating disorder,⁶ or with clinically diagnosed histories of binge eating¹⁰ as well as to variations in the severity of symptoms of emotional eating in women with a clinically diagnosed histories of binge eating¹⁰ or in females in a community sample.⁹ Thus, this model may provide a useful platform for investigation of a clinically relevant aspect of dysregulated eating in human females. There are, however, important differences between the cyclic effects in humans and rats that require further investigation. First, the binge

measure in rats was binge size, whereas the measures in humans were binge frequency or the severity of symptoms of dysregulated eating. Whether the size of binges or bouts of emotional eating vary through menstrual cycle is unknown. Because the neural controls of the frequencies and of the sizes of binge or binge-like eating are likely to differ, this is a potentially crucial issue for mechanistic studies.

The second difference is that the cycle phases during which changes occur in binge or binge-like eating in rats and humans are not fully comparable. In the finest-grain analysis done to date in women¹⁰, binge frequency was lowest during the peri-ovulatory and late-luteal, or premenstrual, phases of the cycle. The 4 d peri-ovulatory phase in women includes the period of maximal estrogen secretion^{33,44} and is similar to the rat estrous phase in terms of the occurrence of ovulation and minimal spontaneous eating^{33,44,45}. Estrus, however, follows the increase in endogenous estrogens that occurs during diestrus 2 and peaks in proestrus^{43,45}. The delay is thought to be due to the latency for the expression of estrogen-controlled genes. Thus, the peri-ovulatory and estrous decreases in bingeing and binge-like eating are both consistent with an estrogenic effect. In contrast, during the late-luteal phase, when binge frequency was also high, estrogen and progestin secretion decrease toward their minima. The most comparable phase in rats is diestrus 1, but during diestrus 1, rats' binge-like eating is high. The explanation for this apparent difference is unclear. Another issue concerns the early and mid-luteal phase. Consistently across all studies,^{4-6,9,10} women's binge frequency was highest in the early to mid-luteal phase, when the corpora lutea secrete increasing amounts of estrogens and progestins.^{33,44} There is no comparable discrete post-ovulatory phase in rats.^{33,45} In rats, successive cycles overlap, and corpora lutea secretion of estrogens and progestins occurs during diestrus 2, i.e., when the ovarian secretions of the next cycle have begun. The relative degrees of estrogen and progestin secretion are also less in diestrus 2 in comparison with the mid-luteal phase. In the present experiment, binge-like eating was similar during diestrus 2, diestrus 1, when both estrogen and progestin secretion are minimal, and proestrus, when ovarian estrogen secretion is higher, but progestin secretion is again minimal. Understanding these species differences is prerequisite to further development of the rat model of the neuroendocrinology of binge eating.

Effects of ovarian hormones in OVX rats

We also determined that E2 treatment was sufficient to suppress binge-like eating in OVX rats (*Experiment 3*) in the cyclic food deprivation/frustrative-nonreward model. This appears to correspond closely to Klump et al.'s¹⁰ finding, based on analyses of binge and hormonal dynamics during the menstrual cycle, of an independent estrogenic inhibition of binge frequency in women, subject to the caveats discussed above. Klump et al.¹⁰, however, also found evidence for a pronounced interactive, inhibitory effect of estrogens and progestins on binge frequency. It is not clear if such an interactive effect occurs in rats. Yu et al.^{24,25} found that binge-like eating in OVX rats was not affected by progesterone alone and was similarly reduced by E2 plus progesterone and by E2 alone in OVX rats, which fails to support a role for progestins in their model. It may be that progestins are simply not involved in the control of binge-like eating in rats. Alternatively, the involvement of dieting and frustrative-nonreward stress in the present model may unveil effects of progestins that were not apparent in Yu et al.'s^{24,25} intermittent access-alone model. In addition, Yu et al. al.^{24,25}

used a near physiological cyclic regimen of progesterone treatment, and previous data indicate that, although physiological progesterone treatment does not affect normal eating in rats,³³ chronic progesterone treatment in pharmacological doses can reverse the eating-inhibitory effects of E2 and stimulates eating in OVX rats.^{46,47} This suggests that it may be possible to model the effects of progestins on emotional and binge eating in humans using chronic, high-dose progesterone treatment in OVX rats.

Negative valence and arousal

The open-field test in *Experiment 2* examined whether binge-like eating induced by intermittent access to palatable food and frustrative-nonreward stress is associated with anxiety, fear or other negative-valence states that lead to reductions in central-zone locomotion and central-zone entries.^{34–36} That neither of these measures decreased in rats that displayed binge-like eating suggests that binge-like eating did not result from increased negative valence. This complements the report of Cao et al.¹⁷ that binge-like eating induced by intermittent access to palatable food did not increase anxiety-like behavior measured by light-dark preference in an elevated plus maze. Thus, further research is required to determine whether the role of negative valence in binge and emotional eating in humans can be modeled in animals.

The increase in central zone locomotion in non-estrous rats subjected to frustrative-nonreward stress and the increase total open-field locomotion in non-estrous rats subjected to food restrictions and frustrative-nonreward stress suggest that increased binge-like eating in this model is associated with general arousal.⁴⁸ That increases in locomotor activity occurred only in non-estrous rats contrasts sharply to the marked increase in spontaneous locomotor activity that normally occurs during estrus.⁴⁹ Further research is warranted to determine whether the arousal is an expression of increased activity of the negative-valence system or whether it contributes causally to binge-like eating. It may be that orexin is involved because orexin stimulates locomotor activity⁵⁰ and administration of an orexin-1 receptor antagonist reduced binge-like eating in this model.⁵¹

Brain mechanisms

Little is known about the neural mechanisms mediating binge-like eating in rodent models. Using transgenic methods Cao et al.¹⁷ found that estrogens act via estrogen receptor-1 (Esr1, formerly ER α) in serotonergic neurons in the dorsal raphe nuclei to reduce binge-like eating produced by intermittent access to palatable food in mice. This implicates serotonin in the inhibitory effect of estrogens on binge-like eating and extends previous reports that neuropharmacological manipulation of serotonergic neurotransmission influences binge-like eating^{26,52,53}, as well as clinical data implicating serotonergic neurotransmission in binge eating.^{12,54}

Micioni Di Bonaventura et al.³¹ reported that pharmacological manipulation of corticotropin-releasing factor (CRF) in the BNST decreased binge-like eating induced by the cyclic food restriction/frustrative-nonreward procedure and that frustrative-nonreward stress increased the numbers of neurons expressing Fos, a marker of neuronal activity, in the dorsal and ventral BNST of non-estrous rats. This involvement of BNST CRF in binge-like eating

suggests that extrahypothalamic CRF systems may control binge eating independent of the hypothalamic-pituitary-adrenal (HPA) axis. The present BNST pERK data extend these findings by suggesting that the neural activation of the BNST of OVX rats subjected to cyclic food restriction and frustrative nonreward is negatively regulated by estrogens. The mechanisms mediating this regulation are not clear, although the delay between E2 treatment and testing suggests that gene regulation may be involved, as is the case for many other estrogenic effects. It is also not clear whether estrogens act directly in the BNST, which expresses *Esr1* abundantly⁵⁵, to exert this inhibitory influence on neuronal activation or whether they act indirectly, for example in the dorsal raphe. Although BNST CRF is not linked to the HPA axis, a role for the HPA axis in binge-like eating after cyclic food restriction/frustrative-nonreward cannot be excluded. This is because frustrative-nonreward [with or without food-restriction history] increased plasma corticosterone levels²⁶ and glucocorticoids selectively stimulate intake of fat and sweet foods.⁵⁶

pERK expression also increased in the PVN and CeA in OVX rats subjected to cyclic food restriction and frustrative nonreward that were not E2 treated, but not in those that were E2 treated; i.e., paralleled induction of binge-like eating. These data add two novel sites to the neuronal network mediating binge-like eating. The decreases in pERK expression in the PVN in E2-treated rats subjected to cyclic food restriction and frustrative-nonreward stress appears similar to the effects of E2 to decrease Fos expression in the PVN in response to restraint stress.⁵⁷ E2, however, failed to affect Fos expression in the CeA in response to noise stress.⁵⁸ The nature of the contributions of the CeA and PVN to binge-like eating cannot be identified at present due to the numerous functional roles of these sites, in particular, their contributions to both the negative-valence system and the control of normal eating. Importantly, our data suggest that E2 modulates these contributions oppositely. That is, E2 increased the numbers of PVN and CeA neurons expressing Fos in response to eating,⁵⁹ whereas E2 decreased the numbers of PVN and CeA neurons expressing pERK in response to cyclic food restriction and frustrative nonreward. This distinction should be useful in identifying the particular PVN and CeA neurons involved in binge-like eating.

Conclusion

These data suggest that binge-like eating elicited by cyclic food-restriction and frustrative-nonreward stress in female rats provides an appropriate model for mechanistic studies of the estrogenic modulation of emotional and binge-like eating in during the menstrual cycle. Furthermore, they suggest that anxiety as measured in the open field does not contribute to binge-like eating under these conditions. Finally, the pERK findings suggest that the PVN, BNST and CeA contribute to the neural mechanism underlying binge-like eating in ways that are distinct from their roles in normal eating.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Italian Ministry of University and Research grants FIRB-RBFR12DELS and PRIN-2012JTX3KL to CC, by IBRO-InEurope Short Stay Grants Program to MVMD, and National Institutes of Health (USA) grant R01 DK092608 to LA, TAL.

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5. Arlington, VA USA: American Psychiatric Association; 2013.
2. Hudson JI, Hiripi E, Pope HG Jr, Kessler RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biol Psychiatry*. 2007; 61:348–358. [PubMed: 16815322]
3. Swanson SA, Crow SJ, Le Grange D, Swendsen J, Merikangas KR. Prevalence and correlates of eating disorders in adolescents. Results from the national comorbidity survey replication adolescent supplement. *Arch Gen Psychiatry*. 2011; 68:714–23. [PubMed: 21383252]
4. Culbert KM, Racine SE, Klump KL. Hormonal factors and disturbances in eating disorders. *Curr Psychiatry Rep*. 2016; 18:65. [PubMed: 27222139]
5. Edler C, Lipson SF, Keel PK. Ovarian hormones and binge eating in bulimia nervosa. *Psychol Med*. 2007; 37:131–141. [PubMed: 17038206]
6. Schoofs N, Chen F, Bräunig P, Stamm T, Krüger S. Binge eating disorder and menstrual cycle in unmedicated women with bipolar disorder. *J Affect Disord*. 2011; 129:75–78. [PubMed: 20869775]
7. Masheb RM, Grilo CM. Emotional overeating and its associations with eating disorder psychopathology among overweight patients with binge eating disorder. *Int J Eat Disord*. 2006; 39:141–146. [PubMed: 16231349]
8. van Strien T. Ice-cream consumption, tendency toward overeating, and personality. *Int J Eat Disord*. 2000; 28:460–464. [PubMed: 11054795]
9. Klump KL, Keel PK, Racine SE, Burt SA, Neale M, Sisk C, Boker S, Hu JY. The interactive effects of estrogen and progesterone on changes in emotional eating across the menstrual cycle. *J Abnorm Psychol*. 2013; 122:131–137. [PubMed: 22889242]
10. Klump KL, Racine SE, Hildebrandt B, Burt SA, Neale M, Sisk CL, Boker S, Keel PK. Ovarian hormone influences on dysregulated eating: A comparison of associations in women with versus without binge episodes. *Clin Psychol Sci*. 2014; 2:545–559. [PubMed: 25343062]
11. Van Strien T, Frijters JE, Bergers GP, Defares PB. The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *Int J Eating Dis*. 1986; 5:295–315.
12. Frank GK, Kaye WH. Current status of functional imaging in eating disorders. *Int J Eat Disord*. 2012; 45:723–736. [PubMed: 22532388]
13. Mathes WF, Brownley KA, Mo X, Bulik CM. The biology of binge eating. *Appetite*. 2009; 52:545–53. [PubMed: 19501749]
14. Treasure J, Claudino AM, Zucker N. Eating disorders. *Lancet*. 2010; 13(375):583–93.
15. Corwin RL, Avena NM, Boggiano MM. Feeding and reward: perspectives from three rat models of binge eating. *Physiol Behav*. 2011; 25(104):87–97.
16. Lutter M, Croghan AE, Cui H. Escaping the golden cage: Animal models of eating disorders in the post-diagnostic and statistical manual era. *Biol Psychiatry*. 2016; 79:17–24. [PubMed: 25777657]
17. Cao X, Xu P, Oyola MG, Xia Y, Yan X, Saito K, Zou F, Wang C, Yang Y, Hinton A Jr, Yan C, Ding H, Zhu L, Yu L, Yang B, Feng Y, Clegg DJ, Khan S, DiMarchi R, Mani SK, Tong Q, Xu Y. Estrogens stimulate serotonin neurons to inhibit binge-like eating in mice. *J Clin Invest*. 2014; 124:4351–62. [PubMed: 25157819]
18. Corwin RL, Wojnicki FH, Fisher JO, Dimitriou SG, Rice HB, Young MA. Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. *Physiol Behav*. 1998; 65:545–53. [PubMed: 9877422]

19. Cottone P, Sabino V, Steardo L, Zorrilla EP. Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. *Neuropsychopharmacol.* 2008; 33:524–535.
20. Czyzyk TA, Sahr AE, Statnick MA. A model of binge-like eating behavior in mice that does not require food deprivation or stress. *Obesity.* 2010; 18:1710–1717. [PubMed: 20300082]
21. Dimitriou SG, Rice HB, Corwin RL. Effects of limited access to a fat option on food intake and body composition in female rats. *Int J Eat Disord.* 2000; 28:436–445. [PubMed: 11054791]
22. Klump KL, Racine S, Hildebrandt B, Sisk CL. Sex differences in binge eating patterns in male and female adult rats. *Int J Eat Disord.* 2013; 46:729–736. [PubMed: 23625647]
23. Murray SM, Tulloch AJ, Chen EY, Avena NM. Insights revealed by rodent models of sugar binge eating. *CNS Spectr.* 2015; 20:530–536. [PubMed: 26510689]
24. Yu Z, Geary N, Corwin RL. Ovarian hormones inhibit fat intake under binge-type conditions in ovariectomized rats. *Physiol Behav.* 2008; 95:501–507. [PubMed: 18706435]
25. Yu Z, Geary N, Corwin RL. Individual effects of estradiol and progesterone on food intake and body weight in ovariectomized binge rats. *Physiol Behav.* 2011; 104:687–693. [PubMed: 21801735]
26. Cifani C, Polidori C, Melotto S, Ciccocioppo R, Massi M. A preclinical model of binge eating elicited by yo-yo dieting and stressful exposure to food: effect of sibutramine, fluoxetine, topiramate, and midazolam. *Psychopharmacol.* 2009; 204:113–125.
27. Cifani, C., Micioni Di Bonaventura, MV., Ciccocioppo, R., Massi, M. Binge eating in female rats induced by yo-yo dieting and stress. In: Avena, NM., editor. *Animal Models of Eating Disorders.* Berlin, Germany: Springer Verlag; 2013. p. 27-49.
28. Sanislow CA, Pine DS, Quinn KJ, Kozak MJ, Garvey MA, Heinssen RK, Wang PS, Cuthbert BN. Developing constructs for psychopathology research: research domain criteria. *J Abnorm Psychol.* 2010; 119:631–639. [PubMed: 20939653]
29. Vannucci A, Nelson EE, Bongiorno DM, Pine DS, Yanovski JA, Tanofsky-Kraff M. Behavioral and neurodevelopmental precursors to binge-type eating disorders: support for the role of negative valence systems. *Psychol Med.* 2015; 45:2921–36. [PubMed: 26040923]
30. Cifani C, Micioni Di Bonaventura MV, Vitale G, Ruggieri V, Ciccocioppo R, Massi M. Effect of salidroside, active principle of *Rhodiola rosea* extract, on binge eating. *Physiol & Behav.* 2010; 101:555–62. [PubMed: 20837037]
31. Micioni Di Bonaventura MV, Ciccocioppo R, Romano A, Bossert JM, Rice KC, Ubaldi M, St Laurent R, Gaetani S, Massi M, Shaham Y, Cifani C. Role of bed nucleus of the stria terminalis corticotrophin-releasing factor receptors in frustration stress-induced binge-like palatable food consumption in female rats with a history of food restriction. *J Neurosci.* 2014; 34:11316–11324. [PubMed: 25143612]
32. Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, Young E. Strategies and methods for research on sex differences in brain and behavior. *Endocrinol.* 2005; 146:1650–1673.
33. Asarian L, Geary N. Sex differences in the physiology of eating. *Am J Physiol: Regul Integr Comp Physiol.* 2013; 305:R1215–1267. [PubMed: 23904103]
34. Bailey, KR., Crawley, JN. Anxiety-Related Behaviors in Mice. In: Buccafusco, JJ., editor. *Methods of Behavior Analysis in Neuroscience.* 2. Vol. chapter 5. Boca Raton, FL USA: CRC Press/Taylor & Francis; 2009.
35. Rayce JR. On the construct validity of open field measures. *Psychol Bull.* 1997; 84:1098–1106.
36. Thiel CM, Muller CP, Huston JP, Schwarting RKW. High versus low reactivity to a novel environment: behavioral, pharmacological and neurochemical assessment. *Neurosci.* 1999; 93:243–251.
37. Asarian L, Geary N. Cyclic estradiol treatment normalizes body weight and restores physiological pattern of spontaneous feeding and sexual receptivity in ovariectomized rats. *Horm Behav.* 2002; 42:461–471. [PubMed: 12488112]
38. Rosen LB, Ginty DD, Weber MJ, Greenberg ME. Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. *Neuron.* 1994; 12:1207–1221. [PubMed: 8011335]

39. Xia Z, Dudek H, Miranti CK, Greenberg ME. Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *J Neurosci*. 1996; 16:5425–5436. [PubMed: 8757255]
40. Micioni Di Bonaventura MV, Vitale G, Massi M, Cifani C. Effect of Hypericum perforatum Extract in an Experimental Model of Binge Eating in Female Rats. *J Obes*. 2012; 2012:956137. [PubMed: 22997570]
41. Micioni Di Bonaventura MV, Cifani C, Lambertucci C, Volpini R, Cristalli G, Massi M. A2A adenosine receptor agonists reduce both high-palatability and low-palatability food intake in female rats. *Behav Pharmacol*. 2012; 23:567–74. [PubMed: 22743606]
42. Micioni Di Bonaventura MV, Ubaldi M, Liberati S, Ciccocioppo R, Massi M, Cifani C. Caloric restriction increases the sensitivity to the hyperphagic effect of nociceptin/orphanin FQ limiting its ability to reduce binge eating in female rats. *Psychopharmacology*. 2013; 228:53–63. [PubMed: 23455592]
43. Pucci M, Micioni Di Bonaventura MV, Giusepponi ME, Romano A, Filaferro M, Maccarrone M, Ciccocioppo R, Cifani C, D'Addario C. Epigenetic regulation of nociceptin/orphanin FQ and corticotropin-releasing factor system genes in frustration stress-induced binge-like palatable food consumption. *Addict Biol*. 2015; doi: 10.1111/adb.12303.
44. Zeleznik, AJ., Pohl, CR. Control of follicular development, corpus luteum function, the maternal recognition of pregnancy, and the neuroendocrine regulation of the menstrual cycle in higher primates. In: Niell, JD., editor. *Knobil and Neill's Physiology of Reproduction*. 3. Amsterdam, The Netherlands: Elsevier/Academic; 2006. p. 2449-2510.
45. Freeman, ME. Neuroendocrine control of the ovarian cycle of the rat. In: Niell, JD., editor. *Knobil and Neill's Physiology of Reproduction*. 3. Amsterdam, The Netherlands: Elsevier/Academic; 2006. p. 2327-2388.
46. Grueso E, Rocha M, Puerta M. Plasma and cerebrospinal fluid leptin levels are maintained despite enhanced food intake in progesterone-treated rats. *Eur J Endocrinol*. 2001; 144:659–665. [PubMed: 11375801]
47. Wade GN. Some effects of ovarian hormones on food intake and body weight of female rats. *J Comp Physiol Psychol*. 1975; 88:183–193. [PubMed: 1120795]
48. Quinkert AW, Vimal V, Weil ZM, Reeke GN, Schiff ND, Banavar JR, Pfaff DW. Quantitative descriptions of generalized arousal, an elementary function of the vertebrate brain. *Proc Natl Acad Sci USA*. 2011; 13(108 Suppl 3):15617–15623.
49. Eckel LA, Houtp TA, Geary N. Spontaneous meal patterns in female rats with and without access to running wheels. *Physiol Behav*. 2000; 70:397–405. [PubMed: 11006440]
50. Butterick TA, Billington CJ, Kotz CM, Nixon JP. Orexin: pathways to obesity resistance? *Rev Endocr Metab Disord*. 2013; 14:357–64. [PubMed: 24005942]
51. Piccoli L, Micioni Di Bonaventura MV, Cifani C, Costantini VJ, Massagrande M, Montanari D, Martinelli P, Antolini M, Ciccocioppo R, Massi M, Merlo-Pich E, Di Fabio R, Corsi M. Role of orexin-1 receptor mechanisms on compulsive food consumption in a model of binge eating in female rats. *Neuropsychopharmacology*. 2012; 37:1999–2011. [PubMed: 22569505]
52. Chandler-Laney PC, Castañeda E, Viana JB, Oswald KD, Maldonado CR, Boggiano MM. A history of human-like dieting alters serotonergic control of feeding and neurochemical balance in a rat model of binge-eating. *Int J Eat Disord*. 2007; 40:136–42. [PubMed: 17080436]
53. Placidi RJ, Chandler PC, Oswald KD, Maldonado C, Wauford PK, Boggiano MM. Stress and hunger alter the anorectic efficacy of fluoxetine in binge-eating rats with a history of caloric restriction. *Int J Eat Disord*. 2004; 36:328–41. [PubMed: 15478135]
54. Hildebrandt T, Alfano L, Tricamo M, Pfaff DW. Conceptualizing the role of estrogens and serotonin in the development and maintenance of bulimia nervosa. *Clin Psychol Rev*. 2010; 30:655–68. [PubMed: 20554102]
55. Leite C, Madeira MD, Sá SI. Effects of sex steroids and estrogen receptor agonists on the expression of estrogen receptor alpha in the principal division of the bed nucleus of the stria terminalis of female rats. *Brain Res*. 2014; 1582:99–106. [PubMed: 25087654]
56. Dallman MF. Stress-induced obesity and the emotional nervous system. *Trends Endocrinol Metab*. 2010; 21:159–65. [PubMed: 19926299]

57. Figueiredo HF, Ulrich-Lai YM, Choi DC, Herman JP. Estrogen potentiates adrenocortical responses to stress in female rats. *Am J Physiol Endocrinol Metab.* 2007; 292:E1173–E1182. [PubMed: 17179393]
58. Dayas CV, Xu Y, Buller KM, Day TA. Effects of chronic oestrogen replacement on stress-induced activation of hypothalamic-pituitary-adrenal axis control pathways. *J Neuroendocrinol.* 2000; 12:784–94. [PubMed: 10929091]
59. Eckel LA, Geary N. Estradiol treatment increases feeding-induced c-Fos expression in the brains of ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol.* 2001; 281:R738–46. [PubMed: 11506987]

Fig. 1. Binge-like eating procedure

Rats were food restricted (66% of preceding *ad libitum* chow intake) or fed *ad libitum* on d 1–4, 9–12, and 17–20. All rats were fed *ad libitum* on d 5–8, 13–16, and 21–24 and offered access to palatable food from 10.00–12.00 h on d 5, 6, 13 and 14. On d 25 rats were subjected to frustrative-nonreward stress for 15 min (10.00–10.15 h) by hanging a cup containing the palatable food inside a metal grid container, so that the rats could see and smell it, but could not obtain it. During this period, rats repeatedly attempted to reach the palatable food. Finally, for the binge-like eating test the cup of palatable-food was placed inside the cage for 2 h (10.15–12.15 h). Control rats were not food restricted, not subjected to stress, or neither. Stressed and non-stressed rats were housed in different rooms to avoid subjecting the non-stressed rats to the smell of the palatable food during the stress procedure.

Fig. 2. Cyclic changes in binge-like eating in intact female rats

A history of intermittent food restriction and a single 15 min period of frustrative-nonreward stress prior to the feeding test increased palatable food intake in female rats tested during diestrus or proestrus, but not in rats tested during estrus (*Experiment 1*). Left: palatable-food intake during the first 15 min of testing; Right: palatable-food intake during the entire 2 h test; kcal/kg body weight, mean $\pm$ SEM. **Different from non-restricted or non-stressed rats, $p < 0.01$; ##Different from rats not in estrus, $p < 0.01$; $n = 17-22$ rats in diestrus or proestrus, $n = 6-8$ rats in estrus.

Fig. 3. Cyclic changes in total distance traveled in intact female rats

Independent of food-restriction history, 15 min of frustrative-nonreward stress increased total distance traveled in the central zone of an open-field in intact female rats tested during diestrus or proestrus, but not in rats tested during estrus (*Experiment 2*). Left: Total distance travelled in the central zone; Right: central-zone entries; mean $\pm$ SEM. *Different from non-restricted or non-stressed groups, $p < 0.05$, ** $p < 0.01$; ##Different from rats not in estrus, $p < 0.01$; $n = 22$ – 24 rats in diestrus or proestrus, $n = 6$ – 7 rats in estrus.

Fig. 4. Estradiol (E2) treatment suppressed binge-like eating in ovariectomized (OVX) rats
 A history of intermittent food restriction and a single 15 min period of frustrative-nonreward stress prior to the binge-eating test increased palatable food intake in OVX rats that did not receive E2 treatment, but not in OVX rats that were treated with E2 (*Experiment 3*). **A**, body weights, g; **B**, palatable-food intake, kcal/kg body weight, during the first 15 min of testing (left) and during the entire 2 h test (right); mean $\pm$ SEM. **Different from non-restricted/non-stressed group, $p < 0.01$; Different from OIL group, # $p < 0.05$, ## $p < 0.01$; $n = 6-7$ per group.

Fig. 5. Estradiol (E2) treatment reduced brain activation elicited by the binge-eating procedure in the central nucleus of the amygdala (CeA) and paraventricular nucleus (PVN), but not in the arcuate nucleus (Arc)

A single 15 min period of frustrative-nonreward stress increased the numbers of cells expressing phosphorylated extracellular signal-regulated kinases (pERK), a marker of neuronal activation, in the brains of ovariectomized (OVX) female rats with a history of intermittent food restriction that received OIL treatment, but not in non-restricted rats or food-restricted rats that were treated with E2 (*Experiment 4*). A, CeA; B, Arc; C, PVN. In each panel, mean $\pm$ SEM numbers of pERK-positive nuclei are shown on left and representative photomicrographs shown on the right. **Different from non-restricted/non-stressed group, $p < 0.01$; ##Different from OIL group, $p < 0.01$; $n = 6-7$ per group.

Fig. 6. Estradiol (E2) treatment reduced brain activation elicited by the binge-eating procedure in the bed nucleus of the stria terminalis (BNST)

A single 15 min period of frustrative-nonreward stress increased the numbers of cells expressing phosphorylated extracellular signal-regulated kinases (pERK), a marker of neuronal activation, in the brains of ovariectomized (OVX) female rats with a history of intermittent food restriction that received OIL treatment, but not in non-restricted rats or food-restricted rats that were treated with E2 (*Experiment 4*). A, dorsal BNST; B, ventral BNST. In each panel, mean $\pm$ SEM numbers of pERK-positive nuclei are shown on left and a representative photomicrographs are shown on the right. . The light area at the top of the ventral BNST photomicrograph from a food-restricted and stressed OIL rat is the anterior commissure; it is not visible in the other sections due to the lack of pERK staining and low background (the ventral BNST was localized using landmarks visible in lower-power images). *Different from non-restricted/non-stressed group, $p < 0.05$, ** $p < 0.01$; #Different from OIL group, $p < 0.05$, ## $p < 0.01$; $n = 6-7$ per group.