Intracerebroventricular Administration of Leptin Increases Anxiety-like Behavior in Female Rats after Semi-starvation: Implications for Anxiety in Eating Disorders

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Abstract

Background

Patients with eating disorders often exhibit abnormal eating conditions like food restriction, adipocyte and body weight reduction, and pathologic anxiety-like behavior. The role of leptin, which is recognized as an adipocyte-derived hormone, on anxiety-like behavior in eating disorders is still unclear.

Methods

We investigated the role of leptin on anxiety-like behavior with or without semi-starvation using the elevated plus-maze test in adolescent female rats. In our first experiment, anxiety-like behavior was evaluated with the elevated plus-maze test 30 min after intracerebroventricular administration of 3 μg of leptin or vehicle. In our second experiment, the rats were allowed access to food for only 2 hr each day for 7 days. Then, leptin or vehicle was administered to the rats after the last 2 hr feeding period, and anxiety-like behaviors were evaluated in the same way as in the first experiment.

Results

In the first experiment, there was no difference between the anxiety-like behaviors observed after leptin administration and those seen after vehicle administration. Under the conditions of semi-starvation, however, the percentage of time spent in the open arms in the rats given leptin was lower than that in rats given vehicle.

Conclusions

These results suggest that leptin administration causes anxiety-like behavior only after semi-starvation. Leptin might play an important role in pathologic anxiety-like behavior in eating disorders.

Key Words: Leptin; Anorexia nervosa; Anxiety; Semi-starvation; Female rat

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Introduction

Eating disorders most commonly begin during adolescence in females. Eating-disorder patients (ED) usually restrict their diet because of an excessive interest in body weight and shape, and believe themselves to be too fat even when they are severely underweight. ED often exhibit other psychological disorders including depression and anxiety, and a high comorbidity with anxiety disorder has been observed[1-3]. Such psychological changes cause and enhance the harmful eating behavior of ED. As the etiology of the eating disorder involves a complex interaction between genetic, environmental, social, and cultural factors, it is difficult to know whether anxiety symptoms cause anorexic behavior or if maintaining chronic dietary restrictions induces anxiety. On a neurochemical level, eating disorders and anxiety disorders share a number of functional abnormalities with respect to their effects on serotonin and other neurotransmitter systems[4]. Thus, food restriction might change the functional activity of these systems, leading to lasting psychological and behavioral alterations[5].

In eating disorders, especially in anorexia nervosa (AN), extreme body weight loss leads to a decrease in adipose tissue and circulating leptin, which is derived primarily from white adipose tissue. Leptin has multiple effects[6-8] such as acting as a lipostatic negative feedback signal to regulate energy balance. Increased circulating leptin levels induce its anorectic actions in the brain[9,10]. In past studies, semi-starvation produced reductions in anxiety-like behavior in rats[11]. The reduction of leptin as a consequence of body weight loss may have an important role in anxiety-like behavior during stressful states like semi-starvation. However, the neuropsychological mechanism that explains the observed comorbidity between eating disorders and anxiety disorders is still unclear.

The purpose of the present study was to determine the effect of leptin on anxiety-like behavior in adolescent female rats with or without semi-starvation (2 hr access to food per day for a week). Intracerebroventricular (i.c.v.) administration of leptin was performed and anxiety-like behavior was evaluated with the elevated plus-maze test (EPM).

Methods

Subjects

For behavioral studies, female Wistar rats (Keari Co., Osaka, Japan) were housed individually in wire-topped, plastic cages (20×30×15 cm) in a vivarium with a 12 hr reversed dark-light cycle (light on 20:00h-8:00h). The vivarium was humidity- and temperature- (22℃) controlled. Rats had access to standard rodent chow (24.8% crude protein, 4.4% crude fat, 3.5% crude fiber, 7.0% crude ash, 51.6% nitrogen-free extract, 8.7% water; CE-2, 345.2 cal/100g: CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum* before food restriction. Rats were cared for in compliance with the Guiding Principles for the Care and Use of Animals and the Guidelines for Animal Experimentation of Osaka City University.

Surgery

Rats were acclimated to the vivarium for at least 1 week and were then implanted with an indwelling cannula directed unilaterally at the left lateral ventricle. Anesthetized (chloral hydrate 400 mg/kg, i.p.) subjects were secured in a stereotaxic frame with the tooth bar set 5.0 mm above interaural zero. A straight, stainless steel, 22-gauge guide cannula (Plastics One Inc., Roanoke, VA) was positioned above the lateral ventricle (the coordinates were −0.6 mm

anterior/posterior, ±2.0 mm lateral to the bregma, and 3.2 mm ventral from the skull surface[14]) and was anchored to the skull with screws and dental cement. A dummy stylet (Plastics One Inc., Roanoke, VA) maintained patency. The subjects were housed individually and allowed 1 week to recover from the surgery.

Drugs and injections

Recombinant rat leptin was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Leptin was dissolved in artificial cerebrospinal fluid (aCSF; 128 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, and 2.6 mM KCl, pH 7.4). The dissolved leptin was divided between several Eppendorf tubes and stored at −80℃ for later use. The stored solution was thawed at room temperature just before the experiment and was used only once. Three microgram of leptin were administered intracerebroventricularly. The dose was selected based on previous studies that indicated that 3 μg of i.c.v. leptin reduced food intake and body weight in rats[12]. I.c.v. administration was performed through a 28-gauge injector that extended beyond the tip of the guide cannula. The injector was attached via polyethylene (PE-20) tubing to a 10 μL Hamilton microsyringe. I.c.v. administrations were conducted over 1 min. The injector was left in place for 1 min after administration to allow diffusion[13].

Elevated plus-maze test

The EPM apparatus consisted of four arms (50 cm long, 10 cm wide). The two closed arms had 40-cm-high dark walls, and the two open arms had none. The illuminance of the lighting in the open arms was 1.5-2.0 lux. The maze was elevated to a height of 50 cm. White noise (70 dB) was present throughout habituation and testing. The rats were placed individually onto the center of the apparatus facing a closed arm during the dark cycle. The time spent in and entries into each arm were detected automatically and recorded by a computer for 5 min (Axis 90, Neuroscience Inc., Osaka, Japan). The percentage of open arm time, as measured by the time spent in the open as opposed to the closed arms, has been proposed to relate inversely to anxiety[15], whereas the number of entries into the closed arms provides an index of locomotor activity[16].

Experimental procedures

Separate groups of rats were tested in the EPM under one of the following two dietary conditions: 1) free feeding or 2) semi-starvation (2 hr access to food after 22 hr food deprivation, repeated for 7 days).

Experiment 1: The effects of leptin on anxiety-like behavior

In the first experiment, rats (n=32; 12 weeks old, 230-280g) were used with the EPM two weeks after their recovery from surgery. Either recombinant rat leptin (3 μg in 5 μL) or 5 μL aCSF was administered to the left lateral ventricle through the guide cannula. After 30 min of i.c.v. administration, the rats were tested with the EPM in the dark phase (15:00h). We measured the time in each arm and entries directed toward each arm to validate the indices of anxiety-like behavior.

Experiment 2: Effects of leptin on anxiety-like behavior during semi-starvation

In the second experiment, rats (n=35; 12 weeks old, 190-240g) underwent time-restricted scheduled feeding (2 hr access per day) for 7 days after a week of surgery. The rats were allowed access to food for 2 hr per day in the dark phase (12:00h-14:00h) and had *ad libitum* access to

water. Body weight was measured during semi-starvation. Either leptin (3 μg in 5 μL) or aCSF was administered intracerebroventricularly after the last 2 hr feeding period of semi-starvation. Then, anxiety-like behavior was measured with the EPM after 30 min of administration.

Data analysis

The body weights of the rats were measured after they had been subjected to the EPM, and those of the leptin and vehicle administration groups were compared in both experiments. The percentage of open arm entries and time spent in the EPM were determined individually. For graphical presentation, the results were expressed as a percentage of the mean, and all results were expressed as the mean±SEM. Statistical comparisons were done using the Student's *t*-test between groups. Differences were considered significant at $p<0.05$. All statistical analyses were carried out using SPSS 11.5J (SPSS, Inc., Chicago, IL).

Histology

After the experiment, the rats were killed with an overdose of diethyl ether. Then, a little ink was injected through the guide cannula. The brains of the subjects were then excised and refrigerated with ice. The chilled brains were cut into coronal sections, and the position of the cannula was verified using the inkblot to see whether it was within the left lateral ventricle. Only data from correctly positioned cannulae were included in the analyses.

Results

The mean±SEM body weights of the rats given leptin and vehicle measured after the EPM were 256.8±10.0g and 253.9±7.3g in experiment 1, and 218.9±4.9g and 220.7±3.7g in experiment 2, respectively. There was no significant difference between the body weights of the two treatment groups in either experiment (p=0.82) (p=0.77) (Table 1).

Table 1. Body weight after the plus-maze test

	Group	n	Body weight (g)
Experiment 1	Leptin	16	256.8±10.0
	Vehicle	16	253.9±7.3
Experiment 2	Leptin	15	218.9±4.9
	Vehicle	20	220.7±3.7

The mean (±SEM) body weights of the rats given leptin and vehicle measured after the plus-maze test. n: the number of rats used.

In experiment 2 body weights of rats were measured at 12:00h and 14:00h, before and after 2hr feeding period, during semi-starvation. Body weights of rats gradually decreased after the onset of food restriction but almost stabilized as measured at 12:00h after 6 days (Fig. 1).

In experiment 1, which examined the effects of leptin on anxiety-like behavior in rats without semi-starvation, there was no significant difference with respect to the percentage of time spent in the open arms [3 μg leptin, 32.59±5.65%; vehicle, 38.70±6.93%; p=0.50] (Fig. 2a) or the number of closed arms entries [3 μg leptin, 13.00±1.00; vehicle, 12.44±0.88; p=0.68] (Fig. 2b) between the 3 μg leptin and vehicle administration groups.

In experiment 2, which was carried out under semi-starvation, the percentage of time spent in

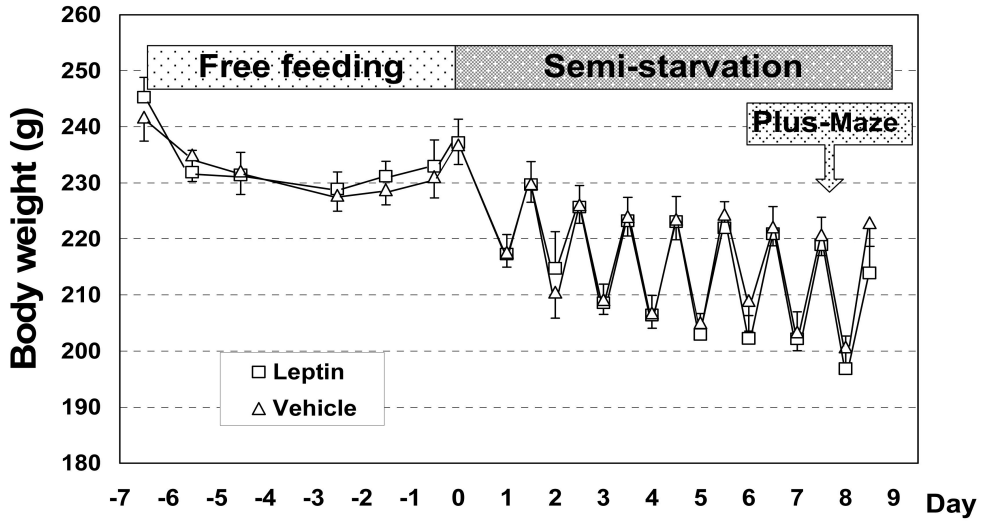

Figure 1. Changes of body weight in the second experiment. Rats (n=35) were acclimated to the vivarium for at least 1 week before undergoing surgery (Day −7). The rats were subjected to time-restricted scheduled feeding for 7 days after the surgery. Body weights of rats were measured before and after 2hr feeding during semi-starvation. On day 7, the rats were tested in the plus-maze after the last 2 hr feeding period.

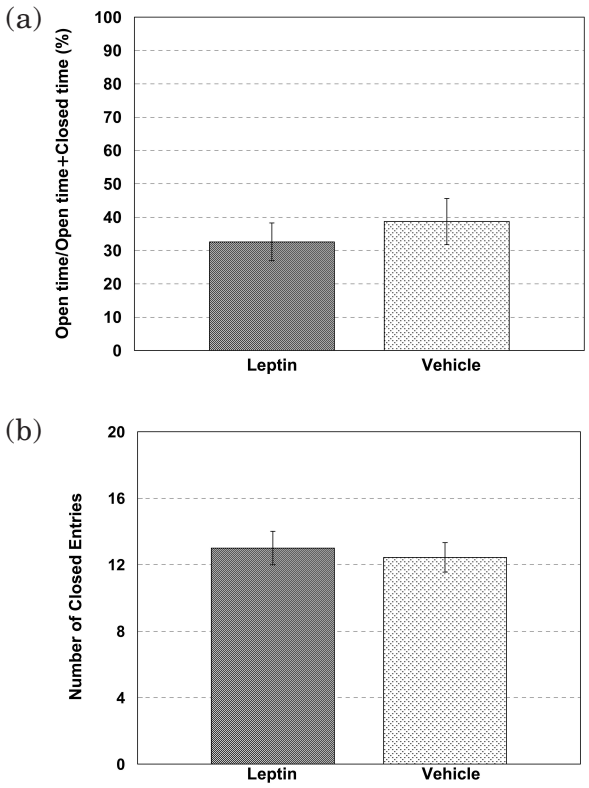

Figure 2. The effects of i.c.v. administration of 3 μg leptin on anxiety-like behavior. Results are expressed as mean±SEM [3 μg leptin, 32.59±5.65%; vehicle, 38.70±6.93%] (a). The effects of an administration of leptin on the number of closed arms entries. Results are expressed as mean±SEM [3 μg leptin, 13.00±1.00; vehicle, 12.44±0.88] (b).

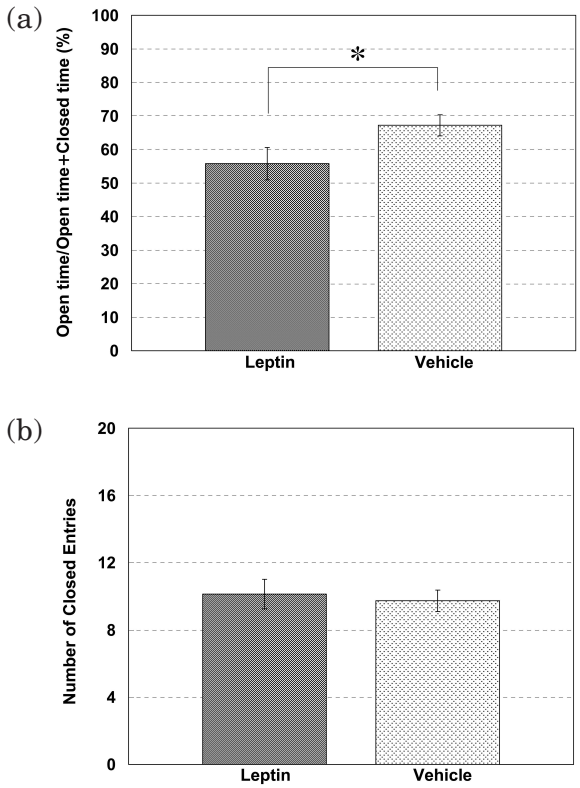

Figure 3. The effects of i.c.v. administration of 3 μg leptin on anxiety-like behavior during semi-starvation. Results are expressed as mean±SEM [3 μg leptin, 55.78±4.83%; vehicle, 67.24±3.13%]. ∗: $p<0.05$ compared with the control group by Student's *t*-test (a). The effects of an administration of leptin on the number of closed arm entries during semi-starvation. Results are expressed as mean±SEM [3 μg leptin, 10.13±0.88; vehicle, 9.74±0.64] (b).

the open arms by the rats that had been administered with 3 μg leptin was significantly lower than that for the rats administered with vehicle [3 μg leptin, 55.78±4.83%; vehicle, 67.24± 3.13%; p=0.045] (Fig. 3a). However, no significant difference in the number of closed arms entries was found [3 μg leptin, 10.13±0.88; vehicle, 9.74±0.64; p=0.719] (Fig. 3b).

Moreover, in comparison to the without semi-starvation group, the semi-starvation group spent a higher percentage of time in the open arms and had a higher number of closed arms entries after both leptin ($p<0.01$) (p=0.04) and vehicle ($p<0.01$) (p=0.016) administrations.

Discussion

In this experiment, anxiety-like behavior was evaluated with the EPM 30 min after i.c.v. administration of leptin or vehicle. There was no difference between the anxiety-like behaviors observed after leptin administration and those observed after vehicle administration without semi-starvation. However, under semi-starvation conditions (access to food for only 2 hr a day for 7 days before treatment) leptin administration induced anxiety-like behavior.

Circulating leptin concentrations are closely related to adipose tissue mass. Plasma leptin concentrations also respond to acute changes in food intake. Leptin levels increase in rodents within hours of a meal[17] and in humans after several days of overfeeding[18], but decrease in both species within hours of the initiation of fasting. Plasma leptin levels rapidly decrease after body weight loss in humans and in rodents[19,20] and can vary between extremely low and being within the low normal range in AN patients[21-23]. In this study, the concentration of leptin in the brain was not measured. From these previous studies, however, a reduction of leptin in the brain of the rats that underwent semi-starvation is speculated. During periods of reduced leptin concentrations in the brain, leptin i.c.v. administration will replenish leptin levels and may affect behavior.

Until recently, there were few reports about the relationship between body weight and anxiety-like behaviors. In this study, rats that had been semi-starved spent more time in open arms and had a higher number of open arms entries compared with *ad libitum* feeding rats receiving either vehicle or leptin groups. This is consistent with previous studies that showed that chronic food restriction reduced anxiety in rats[11]; however, the neurochemical mechanisms of this are still underinvestigation. The decreased leptin concentration observed after semi-starvation might therefore affect anxiety-related behavior. In this study, there was no difference between the anxiety-like behaviors seen after leptin administration and those seen after vehicle administration without semi-starvation. This suggests that leptin has little effect on anxiety-like behavior during normal feeding, but does have an effect after semi-starvation i.e. after leptin reduction, suggesting that leptin affects anxiety-related behavior.

It is reported that leptin treatment increases exploration of the open arms and locomotor activity compared with saline-treatment of ob/ob mice, which have a leptin deficiency, in the EPM[24,25]. These reports are inconsistent with our results. The differences between the rodents (ob/ob mice or rat) and/or the feeding conditions used might be the cause of this. Alternatively, the contrary effects of leptin on anxiety-like behavior might be caused by starvation, a type of stress, which is a common stimulus of the hypothalamic-pituitary-adrenal axis (HPAA)[26,27]. Corticotropin-releasing factor (CRF) mediates neuroendocrine, autonomic, and behavioral responses to stress and seems to play a key role in the aetiology of stress-related psychiatric

diseases such as depression and anxiety[28-31]. Leptin is involved in not only effects on feeding but also in endocrine stress responses that are regulated by glucocorticoids[32]. Previous animal studies have shown that i.p. injected leptin attenuates restraint stress-induced increases in plasma ACTH and corticosterone during starvation[19,33]. Moreover in rats, leptin reduced treadmill running-induced elevation of corticosterone plasma levels and inhibited the induction of the type 1 CRF receptor (CRF_1R) in the paraventricular hypothalamic nucleus, but strongly increased the expression of the type 2 CRF receptor (CRF_2R) in the ventromedial hypothalamic-nucleus[34,35]. Whereas, activation of CRF_1R is clearly anxiogenic, CRF_2R may have a dual role in fear and anxiety-related behavior as well as in the HPAA response to stress[31]. Pharmacological studies have shown that activation of CRF_2R can result in both anxiolytic[36-38] and anxiogenic effects[39,40], which may depend on the specific brain regions involved[41]. CRF_2, which is activated by fasting, might mediate anxiety-like behavior in rats after semi-starvation.

In this experiment, only one dose (3 μg) was applied to rats, and the dose might have been too little to affect some behaviors under normal feeding conditions. However, previous reports have suggested that 3 μg is enough to effect certain behaviors. Administration of i.c.v. leptin altered food intake and body weight dose-dependently from 1 to 30 μg in rats[42]. Moreover, 3.5 μg leptin altered meal parameters[43] and the expression of hypothalamic neuropeptides[44]. I.c.v. injection of 2 μg leptin also inhibited food intake and increased hypothalamic CRH content[45]. We injected 3 μg of leptin intracerebroventricularly. The dose of leptin in this study was selected based on these previous studies. Additional study with other leptin concentrations might clarify the dose-dependent effect of leptin.

As for locomotor activity, chronic intraperitoneal administration of leptin suppressed semi-starvation induced hyperactivity[46]. Additionally, in activity-based anorexia rats, chronic leptin treatment (i.c.v. 4 μg/day) reduced running wheel activity[47]. However in this study, i.c.v. administration of leptin did not influence the number of closed arm entries. The effect of leptin on activity might appear gradually and need continuous administration with or without semi-starvation.

When using female rats, the estrous cycle influences feeding and anxiety-like behavior. It was reported that sex steroid hormones affect the anxiety level of ovariectomized mice[48]. Proestrus female rats spend more time in open arms than diestrus rats[49]. Additionally, under emotional stress the food intake of female rats is inhibited more than that of male rats, and this inhibition is more prominent during proestrus than during the other phases of the estrous cycle[50]. We used female rats because eating disorders are more frequent in females than males. Regardless of the estrous cycle, there were significant differences in anxiety-like behavior; therefore, leptin had strong effects on anxiety during semi-starvation.

AN usually begins with harmless dieting, which then gets out of control. Regardless of their very low body weight, patients with AN desire a lower body weight even though this might lead to death. This excessive dieting is associated with a distorted image of their bodies, with patients believing themselves to be too fat even when they are severely underweight. AN patients have anxiety about gaining weight. We reported that leptin increased anxiety-like behavior only in semi-starvation rats, which have previously been indicated as a model of AN[51]. This might indicate that an increase in plasma leptin in patients with AN leads to resistance to gaining weight when they recover. During recovery, AN patients show hyperleptinemia, as a

result of rapid body weight gain[22,23].

In summary, i.c.v. administration of leptin showed no significant effect on anxiety-like behavior under normal conditions, whereas during semi-starvation it increased anxiety-like behavior in female rats. Leptin might play an important role in anxiety-like behavior during semi-starvation. The increasing leptin level that occurs after gaining weight in ED leads to increased anxiety, and this might be related to the resistance to therapy seen in ED.

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References

1. Fornari V, Kaplan M, Sandberg DE, Mattews M, Skolnick N, Katz J. Depressive and anxiety disorders in anorexia nervosa and bulimia nervosa. Int J Eat Disord 1992;12:21-29.
2. Mitchell JE, Specker SM, de Zwaan M. Comorbidity and medical complications of bulimia nervosa. J Clin Psychiatry 1991;52:13-20.
3. Godart NT, Flament MF, Lecrubier Y, Jeammet P. Anxiety disorders in anorexia nervosa and bulimia nervosa: co-morbidity and chronology of appearance. Eur Psychiatry 2000;15:38-45.
4. Brewerton TD. Toward a unified theory of serotonin dysregulation in eating and related disorders. Psychoneuroendocrinology 1995;20:561-590.
5. Polivy J. Psychological consequences of food restriction. J Am Diet Assoc 1996;96:589-592.
6. Bates SH, Myers MG. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol Metab 2003;14:447-452.
7. Guha PK, Villarreal D, Reams GP, Freeman RH. Role of leptin in the regulation of body fluid volume and pressures. Am J Ther 2003;10:211-218.
8. Harvey J, Ashford ML. Leptin in the CNS: much more than a satiety signal. Neuropharmacology 2003;44: 845-854.
9. Kastin AJ, Pan W, Akerstrom V, Hackler L, Wang C, Kotz CM. Novel peptide-peptide cooperation may transform feeding behavior. Peptides 2002;23:2189-2196.
10. Rivest S. Does circulating leptin have the ability to cross the blood-brain barrier and target neurons directly? Endocrinology 2002;143:3211-3213.
11. Inoue K, Zorrilla EP, Tabarin A, Valdez GR, Iwasaki S, Kiriike N, et al. Reduction of anxiety after restricted feeding in the rat: implication for eating disorders. Biol Psychiatry 2004;55:1075-1081.
12. Choi S, Sparks R, Clay M, Dallman MF. Rats with hypothalamic obesity are insensitive to central leptin injections. Endocrinology 1999;140:4426-4433.
13. Fekete EM, Inoue K, Zhao Y, Rivier JE, Vale WW, Szücs A, et al. Delayed satiety-like actions and altered feeding microstructure by a selective type 2 corticotropin-releasing factor agonist in rats: intra-hypothalamic urocortin 3 administration reduces food intake by prolonging the post-meal interval. Neuropsychopharmacology 2007;32:1052-1068.
14. Pellegrino LJ, Pellegrino AS, Cushman AJ. A stereotaxic atlas of the rat brain. 2nd ed. New York: Plenum Press, 1979.
15. Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol Biochem Behav 1996;54:31-40.
16. Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav 1994;49:171-176.
17. Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, et al. Transient increase in obese gene expression after food intake or insulin administration. Nature 1995;377:527-529.
18. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab 1996;81:4162-4165.
19. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382:250-252.
20. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1:1155-

1161.
21. Calandra C, Musso F, Musso R. The role of leptin in the etiopathogenesis of anorexia nervosa and bulimia. Eat Weight Disord 2003;8:130-137.
22. Hebebrand J, Blum WF, Barth N, Coners H, Englaro P, Juul A, et al. Leptin levels in patients with anorexia nervosa are reduced in the acute stage and elevated upon short-term weight restoration. Mol Psychiatry 1997;2:330-334.
23. Mantzoros C, Flier JS, Lesem MD, Brewerton TD, Jimerson DC. Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. J Clin Endocrinol Metab 1997;82:1845-1851.
24. Picard F, Richard D, Huang Q, Deshaies Y. Effects of leptin adipose tissue lipoprotein lipase in the obese ob/ob mouse. Int J Obes Relat Metab Disord 1998;22:1088-1095.
25. Asakawa A, Inui A, Inui T, Katsuura G, Fujino MA, Kasuga M. Leptin treatment ameliorates anxiety in ob/ob obese mice. J Diabetes Complications 2003;17:105-107.
26. Dallman MF. Stress update Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. Trends Endocrinol Metab 1993;4:62-69.
27. Chowers I, Einat R, Feldman S. Effects of starvation on levels of corticotrophin releasing factor, corticotrophin and plasma corticosterone in rats. Acta Endocrinol 1969;61:687-694.
28. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 1999;160:1-12.
29. Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK, et al. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat Genet 1998;19:162-166.
30. Koob GF, Heinrichs SC. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res 1999;848:141-152.
31. Reul JM, Holsboer F. Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. Curr Opin Pharmacol 2002;2:23-33.
32. De Vos P, Saladin R, Auwerx J, Staels B. Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. J Biol Chem 1995;270:15958-15961.
33. Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology 1997;138:3859-3863.
34. Huang Q, Timofeeva E, Richard D. Regulation of corticotropin-releasing factor and its types 1 and 2 receptors by leptin in rats subjected to treadmill running-induced stress. J Endocrinol 2006;191:179-188.
35. Makino S, Nishiyama M, Asaba K, Gold PW, Hashimoto K. Altered expression of type 2 CRH receptor mRNA in the VMH by glucocorticoids and starvation. Am J Physiol 1998;275:R1138-1145.
36. Valdez GR, Inoue K, Koob GF, Rivier J, Vale W, Zorrilla EP. Human urocortin Ⅱ: mild locomotor suppressive and delayed anxiolytic-like effects of a novel corticotropin-releasing factor related peptide. Brain Res 2002;943:142-150.
37. Valdez GR, Zorrilla EP, Rivier J, Vale WW, Koob GF. Locomotor suppressive and anxiolytic-like effects of urocortin 3, a highly selective type 2 corticotropin-releasing factor agonist. Brain Res 2003;980:206-212.
38. Venihaki M, Sakihara S, Subramanian S, Dikkes P, Weninger SC, Liapakis G, et al. Urocortin Ⅲ, a brain neuropeptide of the corticotropin-releasing hormone family: modulation by stress and attenuation of some anxiety-like behaviours. J Neuroendocrinol 2004;16:411-422.
39. Pelleymounter MA, Joppa M, Ling N, Foster AC. Pharmacological evidence supporting a role for central corticotropin-releasing factor$_2$ receptors in behavioral, but not endocrine, response to environmental stress. J Pharmacol Exp Ther 2002;302:145-152.
40. Pelleymounter MA, Joppa M, Ling N, Foster AC. Behavioral and neuroendocrine effects of the selective CRF2 receptor agonists urocortin Ⅱ and urocortin Ⅲ. Peptides 2004;25:659-666.
41. Radulovic J, Rühmann A, Liepold T, Spiess J. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. J Neurosci 1999;19:5016-5025.
42. Hulsey MG, Lu H, Wang T, Martin RJ, Baile CA. Intracerebroventricular (i.c.v.) administration of mouse leptin in rats: behavioral specificity and effects on meal patterns. Physiol Behav 1998;65:445-455.
43. Flynn MC, Scott TR, Pritchard TC, Plata-Salamán CR. Mode of action of OB protein (leptin) on feeding. Am J Physiol 1998;275:R174-179.
44. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996;98:1101-1106.
45. Uehara Y, Shimizu H, Ohtani K, Sato N, Mori M. Hypothalamic corticotropin-releasing hormone is a mediator of the anorexigenic effect of leptin. Diabetes 1998;47:890-893.
46. Exner C, Hebebrand J, Remschmidt H, Wewetzer C, Ziegler A, Herpertz S, et al. Leptin suppresses semi-

starvation induced hyperactivity in rats: implications for anorexia nervosa. Mol Psychiatry 2000;5:476-481.
47. Hillebrand JJ, Koeners MP, de Rijke CE, Kas MJ, Adan RA. Leptin treatment in activity-based anorexia. Biol Psychiatry 2005;58:165-171.
48. Galeeva A, Tuohimaa P. Analysis of mouse plus-maze behavior modulated by ovarian steroids. Behav Brain Res 2001;119:41-47.
49. Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC. Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiol Behav 2001;74:435-440.
50. Kuriyama H, Shibasaki T. Sexual differentiation of the effects of emotional stress on food intake in rats. Neuroscience 2004;124:459-465.
51. Siegfried Z, Berry EM, Hao S, Avraham Y. Animal models in the investigation of anorexia. Physiol Behav 2003;79:39-45.