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Central Expression and Anorectic Effect of Brain-Derived Neurotrophic Factor Are Regulated by Circulating Estradiol Levels

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Abstract

Estrogens potently suppress food intake. Compelling evidence suggests that estradiol, the primary form of estrogens, reduces food intake by facilitating other anorectic signals. Brain-derived neurotrophic factor (BDNF), like estradiol, appears to suppress food intake by affecting meal size. We hypothesized that estradiol modulates *Bdnf* expression and the anorectic effect of BDNF. The first goal was to determine whether *Bdnf* expression was regulated by endogenous estradiol of cycling rats and by cyclic estradiol treatment using ovariectomized rats. *Bdnf* expression within the ventromedial nucleus of hypothalamus (VMH) was temporally elevated at estrus following the estradiol peak, which coincided with the decline in feeding at this phase of the ovarian cycle. Additionally, food intake and body weight were increased following ovariectomy with a parallel decrease in *Bdnf* expression in the VMH. All of these alterations were reversed by cyclic estradiol treatment, suggesting that *Bdnf* expression within the VMH was regulated in an estradiol-dependent manner. The second goal was to determine whether estradiol modulates the anorectic effect of BDNF. Sham-operated estrous rats and ovariectomized rats cyclically treated with estradiol responded to a lower dose of central administration of BDNF to decrease food intake than male rats and oil-treated ovariectomized rats, implying that endogenous estradiol or cyclic estradiol replacement increased the sensitivity to anorectic effect of BDNF. These data indicate that *Bdnf* expression within the VMH and the anorectic effect of BDNF varied depending on plasma estradiol levels, suggesting that estradiol may regulate BDNF signaling to regulate feeding.

Keywords

ovariectomy; ovarian cycle; food intake; ventromedial nucleus of hypothalamus

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Introduction

The ovarian hormone estradiol, the primary form of estrogens, is not only a hormone essential for reproduction but is also involved in physiological control of energy balance by potently suppressing food intake in many species including humans (Asarian and Geary 2006). In women, caloric intake varies across the menstrual cycle. Women tend to eat less during the four-day periovulatory phase of the menstrual cycle when estradiol reaches its peak (Lissner, Stevens et al. 1988; Buffenstein, Poppitt et al. 1995), and these cyclic changes in feeding are absent in women with anovulatory cycles (Barr, Janelle et al. 1995). Many postmenopausal women gain body weight due to natural decrease in endogenous estradiol levels (Gambacciani, Ciaponi et al. 1997) which can be prevented by estrogen replacement therapy (Gambacciani, Ciaponi et al. 1997), implying a role of estradiol in maintenance of body weight. Similarly, cycling female rodents consume different amounts of food across their four-day ovarian cycles, consuming the least during estrus, which occurs right after preovulatory rise in estradiol secretion, and consuming the most during diestrus when estradiol levels are lower (Tarttelin and Gorski 1971; Asarian and Geary 2002; Asarian and Geary 2006), indicating that physiologic estradiol levels are negatively correlated with food intake (Brobeck, Wheatland et al. 1947; Blaustein and Wade 1976). Disruption of ovarian cycling in rats by ovariectomy (OVX) leads to increases in food intake and weight gain (Wade 1975; Wade and Gray 1979; McElroy and Wade 1987). A cyclic regimen of estradiol replacement at a physiologic dose, designed to mimic the normal changes in plasma estradiol levels across the ovarian cycle, abolishes OVX-induced hyperphagia and normalizes body weight to the levels of gonadally intact rats (Asarian and Geary 2002). Together, available data suggest that estradiol exerts an inhibitory effect on food intake.

Estradiol suppresses feeding by enhancing the potency of other anorectic signals, such as cholecystokinin (Geary 2001) and apolipoprotein A-IV (Shen, Wang et al. 2010), and by decreasing the potency of orexigenic signals such as melanin-concentrating hormone (Messina, Boersma et al. 2006) and ghrelin (Clegg, Brown et al. 2007). Brain-derived neurotrophic factor (BDNF) could be another putative candidate influenced by estradiol. BDNF is one of the important anorectic signals primarily synthesized by VMH in the hypothalamus (Noble, Billington et al. 2011). Acute central administration of BDNF decreases food intake in rats (Wang, Bomberg et al. 2007; Wang, Bomberg et al. 2010). Furthermore, central ventricular BDNF administration suppressed body weight through reductions in meal size and thereby food intake (Spaeth, Kanoski et al. 2012). Mice deficient in BDNF are hyperphagic, and detailed analysis of the feeding patterns of high-fat diet-induced hyperphagia in BDNF deficient mice has revealed that such hyperphagia is mediated by a selective increase in meal size accompanied by a reduced satiety ratio, but no change in meal number (Fox and Byerly 2004). These findings suggest that BDNF, like estradiol, influences feeding by selectively affecting meal termination and satiation, and therefore affecting the control of meal size.

Two nuclear estrogen receptors (ER), ER α and ER β , and one membrane ER GPR 30 have been identified. Available evidence supports that ER α mediates the effects of estradiol on energy balance. ER α is abundantly expressed in the arcuate nucleus and the ventromedial nucleus of hypothalamus (VMH) (Shughrue, Lane et al. 1997; Chakraborty, Hof et al. 2003), two hypothalamic areas associated with feeding and energy homeostasis. In contrast, ER β expression is barely detectable in the arcuate nucleus or VMH (Shughrue and Merchenthaler 2001). Instead, high levels of ER β expression are found in the paraventricular nucleus (PVN) and supraoptic nucleus, the hypothalamic regions with little or no ER α expression (Shughrue, Komm et al. 1996; Shughrue, Lane et al. 1997; Österlund, G.J.M. Kuiper et al. 1998; Shughrue and Merchenthaler 2001). Mice deficient in ER α , both males and females, have increased body weight and adiposity (Heine, Taylor et al. 2000),

whereas mice deficient in ER β have a normal body weight and feeding behavior (Couse and Korach 1999), suggesting a specific role for ER α , but not ER β , in mediating the effects of estradiol on energy balance and feeding. Furthermore, inhibition of ER α by RNA interference in the VMH results in severe obesity and metabolic syndrome (Musatov, Chen et al. 2007), suggesting the importance of ER α within the VMH in mediating energy homeostasis.

Gene expression and immunoreactivity of BDNF, like those of ER α , are distributed at the highest level in the VMH within the hypothalamus (Xu, Goulding et al. 2003), the CNS satiety center that regulates anorexia. *Bdnf* mRNA and BDNF immunoreactivity are also moderately distributed in the medial and ventral parvocellular region of the PVN (Conner, Lauterborn et al. 1997). Blurton-Jones et al. (2004) reported the colocalization of BDNF and ER α in the VMH, suggesting possible interactions between BDNF and estradiol in the regulation of feeding. Thus, the goal of this series of experiments was to determine a possible interaction of estradiol and BDNF in the control of food intake in male and female rats. We hypothesized that *Bdnf* expression and the anorectic effect of BDNF are regulated by estradiol levels. We first tested whether *Bdnf* gene expression at the VMH and the PVN was regulated in an estrogen-sensitive manner. We then examined whether estradiol facilitated the anorectic effect of BDNF in gonadally intact male and female rats and in ovariectomized (OVX) rats with or without estradiol replacement. The results collectively suggested a role for estrogen in regulating the effects of BDNF on feeding.

Materials and Methods

Animals

Adult (12 weeks-old) male and female Long-Evans rats (Harlan, Indianapolis, IN) were housed individually in a temperature-controlled (22 ± 2 °C) vivarium on a 12-h light, 12-h dark cycle (lights on at 0200 h, lights off at 1400 h). Pellet rodent chow (Teklad 7912, Madison, WI) and water were provided *ad libitum* except as otherwise noted. All animal procedures were approved by the Institutional Animal Care and Use Committee of Miami University Ohio, and were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Experiment Procedure

Experiment 1: *Bdnf* gene expression of intact male and female rats—This experiment tested whether *Bdnf* expression was modulated by endogenous estradiol under physiological conditions in intact male rats and in female rats with regular four-day ovarian cycle. Ten naïve male rats and forty naïve female rats were used. Body and food weights of female rats were monitored daily across the ovarian cycle. Daily food intake was the differences in food weights of two consecutive days, and was calculated by subtracting the food weight of the second day from the food weight of the previous day.

Determining stages of ovarian cycle: The stages of the ovarian cycle of female rats were determined by examining the predominant cell types of vaginal cytology samples. Estrus was characterized by large clumps of non-nucleated cornified cells; metestrus was characterized by leukocytes mixed with other cell types; diestrus was characterized by leukocytes without nuclei; and proestrus was characterized primarily by nucleated larger round cells. Female rats that displayed regular four-day ovarian cycles were used in this study. Estrus, the phase when female rats display sexual receptivity following estradiol secretion peak, is the phase when rodents' physiology and behavior are mostly influenced by endogenous estrogens and thus is the phase commonly used to study sex differences (Becker, Arnold et al. 2005).

Ten female rats were sacrificed at each of four phases of the ovarian cycle, proestrus, estrus, metestrus and diestrus (n=10). Male rats (n=10) were sacrificed during the same period. All rats were sacrificed by CO₂ asphyxiation towards the end of light phase, between 1100 h and 1300 h. Trunk blood samples were collected after decapitation and were kept on ice. The plasma was isolated by centrifugation and stored at -80 °C until analyzed. Plasma estradiol level was measured by estradiol radioimmunoassay assay (RIA; Diagnostic Systems Laboratories Inc., Webster, TX, DSL-39100), with intra- and inter-assay coefficients of variation of 3.9–4.1%, and sensitivity of 1.5 pg/mL.

Quantitative real-time PCR for *Bdnf* mRNA level measurement: The brains of the rats were immediately removed after decapitation and frozen at -80 °C until *Bdnf* gene expression was measured by quantitative real time PCR. Briefly, frozen brains were sectioned at 300 µm slices with a cryostat at -15 °C through the frontal plane from rostral to caudal position. The VMH and the PVN were bilaterally microdissected from frozen sections that were approximately relative to Bregma -1.78 to -3.58 mm for the VMH and Bregma -1.08 to -2.16 mm for the PVN with calibrated hollow needles of 0.76 mm in diameter (Stoelting, Kiel, WI), using neuroanatomical landmarks depicted in Swanson (Swanson 1999) as a guide. Total RNA was isolated from microdissected VMH or PVN using RNeasy-micro kit (Ambion, Austin, TX). cDNA was synthesized using an iScript kit (Bio-Rad, Hercules, CA). The constitutively expressed ribosomal protein *L32* was used as an endogenous control to indicate relative quantification of *Bdnf* expression from every sample. The primer sequences for *rattus norvegicus* ribosomal protein *L32* (GenBank accession number NM_013226) from 5' to 3' were forward CAT CGT AGA AAG AGC AGC AC and reverse GCA CAC AAG CCA TCT ATT CAT. The primer sequences for *rattus norvegicus* *Bdnf* (GenBank accession number NM_012513) from 5' to 3' were forward GCG GCA GAT AAA AAG ACT GC and reverse GCA GCC TTC CTT CGT GTA AC. The identity of the amplification products was confirmed by gel electrophoresis and melt curve analysis.

Quantitative PCR reactions were performed using a Bio-Rad iCycler (Bio-Rad, Hercules, CA) and iQ SYBR Green Supermix (Bio-Rad) with 2-step amplification at 95 °C for 10 s and annealing temperature of 58 °C for 30 s for 40 cycles. A standard curve was performed using a dilution series spanning 7 orders of magnitude and samples were measured within the linear part of the standard curve. The PCR efficiencies for *L32* and *Bdnf*, calculated from the slope of the plot threshold cycle (C_T) versus log starting concentration, were between 95–100%. Correlation coefficients for standard curves were between 0.997–1.00. Samples were run in triplicates and normalized to the constitutively expressed ribosomal protein *L32* by calculating the difference of C_T between control gene *L32* and *Bdnf*, ΔC_T (*Bdnf* - *L32*). High ΔC_T (*Bdnf* - *L32*) values represent low expression of *Bdnf*, while low ΔC_T (*Bdnf* - *L32*) values correspond to high *Bdnf* gene expression. ΔC_T (*Bdnf* - *L32*) was used to analyze the correlation between plasma estradiol level and *Bdnf* expression. For relative quantification, the ΔC_T was averaged for the control group from each study and was then subtracted from the ΔC_T of each sample from experimental groups of each study to calculate the approximate fold difference (Applied Biosystems manufacturer's instructions).

Experiment 2: *Bdnf* gene expression of OVX rats with cyclic estradiol replacement—This experiment tested whether *Bdnf* expression was altered following ovariectomy (OVX) with or without exogenous estradiol replacement. Prior to surgery, forty naïve female rats were weight-matched and assigned to one of the four groups (n=10): sham operation with oil vehicle treatment (Sham-Oil), OVX with cyclic estradiol treatment (OVX-E2), OVX with oil treatment and *ad libitum* feeding (OVX-Oil), and OVX with oil treatment and pair-feeding to OVX-E2 group (OVX-Oil PF). Cyclic estradiol or oil

treatment was carried out for five cycles beginning the third day of postoperative recovery. OVX-Oil PF rats were provided with the same amount of food consumed by OVX-E2 rats each day using the pair-feeding method.

OVX or sham surgery: OVX was performed to decrease endogenous estrogen and disrupt the ovarian cycle of female rats. OVX or sham surgery was performed under isofluorane anesthesia (Butler Schein Animal Health, Dublin, OH) using an intra-abdominal approach. Briefly, the ovaries were retracted at the distal end of the oviduct through midline laparotomies and were removed using surgical blades. The distal end of the oviduct and surrounding tissue were clamped with hemostats for two minutes to avoid bleeding. Minimal adipose tissue was removed during the procedure. The musculature was closed with silk sutures and the skin was closed with wound clips. The sham operation procedure for female rats consisted of exposure of the ovaries on both sides but leaving them intact and the same closure of incision as OVX surgery. For male rats, the sham operation procedure consisted of a surgical incision through the abdominal wall and closure of incision.

Cyclic estradiol or oil treatment: Cyclic estradiol or oil treatment was begun on the third day of OVX or sham postoperative recovery when plasma estradiol concentration should have significantly decreased due to rapid degradation of estradiol in the plasma by steroidal esterases (Lund-Pero, Jeppson et al. 1994). 17- β -estradiol-3-benzoate (estradiol; Sigma-Aldrich, St. Louis, MO) was dissolved in sesame oil (Sigma-Aldrich) at a concentration of 0.02 mg/ml. On the third day after OVX, the rats were injected subcutaneously once every fourth day with 2 μ g estradiol in 100 μ l oil vehicle. This regimen of cyclic estradiol treatment has been described to simulate the endogenous fluctuations of estradiol (Asarian and Geary 2002). Specifically this procedure and dose of injection mimics, in both magnitude and duration, the changes in plasma estradiol levels similar to those across the four-day ovarian cycle in gonad-intact female rats (Asarian and Geary 2002), and it decreases food intake the second day after estradiol injection, the day that models estrus (Asarian and Geary 2002). Injection of 100 μ l oil alone to OVX and sham-operated rats was used for control treatment.

Pair-feeding: OVX induces hyperphagia and weight gain (Wade 1975; Wade and Gray 1979; McElroy and Wade 1987). In order to examine the effects of estradiol on *Bdnf* expression and anorectic effect of BDNF without a potential confound of an increase in body weight due to OVX, a group of oil-treated OVX rats were paired (OVX-Oil PF) by being provided with the same amount of food consumed by OVX-E2 rats each day. Using the pair-feeding method, OVX-Oil PF rats had similar body weights as OVX-E2 and sham-operated female rats (unpublished lab observation). During pair-feeding food was weighed daily at 1200 h. OVX-Oil PF rats were supplied food twice a day (towards the end of light phase between 1300 h and 1400 h and four hours later between 1700 h and 1800 h) and were provided with half of the amount of food consumed by the OVX-E2 group each time.

Sham-Oil rats were staged to verify normal ovarian cycling conditions during the experimental period, as described in *Experiment 1*. Sham-Oil rats were injected with oil on proestrous phase. All rats were sacrificed between 1100 h and 1300 h the day after last estradiol or oil injection. Thus Sham-Oil rats were killed during endogenous estrus and OVX-E2 were killed on the day that models estrus (Asarian and Geary 2002). All rats were sacrificed by CO₂ asphyxiation followed by decapitation between 1100 h and 1300 h, and their brains and trunk blood were collected at sacrifice. Plasma estradiol level was measured by estradiol RIA and *Bdnf* gene expressions within the VMH and the PVN were analyzed by quantitative real time PCR, as described in *Experiment 1*.

Experiment 3: Anorectic effect of central BDNF in male and female rats—This experiment examined the effect of BDNF on food intake in sham-operated male and female rats with intact gonads and in OVX rats with or without estradiol treatment. Five groups of rats were used, Male Sham-Oil (n = 8), Female Sham-Oil (n = 8), OVX-Oil (n = 8), OVX-Oil PF (n = 7), and OVX-E2 (n = 7), to test whether estradiol influences the effects of BDNF in feeding suppression. Naïve rats were monitored for body weight, food intake, and ovarian cycle for two weeks after arrival in our animal facility. Rats then received OVX or sham surgery as described in *Experiment 2*, and were implanted with guide cannulas that targeted the third cerebral ventricle.

Cannulation of the third ventricle: Rats were anesthetized with isoflurane (Butler Schein Animal Health) and were implanted with a 22-gauge stainless steel guide cannulas that targeted the third cerebral ventricle. Briefly, rats were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with lambda and bregma at the same vertical coordinate. A 22- gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was lowered on the midline from the dural meninge, 2.2 mm anteroposterior and 7.5 mm dorsoventral with respect to bregma based on the atlas of Paxinos and Watson (Paxinos and Watson 1998). The guide cannulas were fixed to the skull with mounting anchor screws (Plastics One) and dental acrylic. Removable obturators (Plastics One) that extended 0.5 mm beyond the tip of the guide cannulas were inserted into the guide cannula and secured. Cannula placement was verified after one-week postsurgical recovery.

After one week of postoperative recovery, cannula placement was verified by monitoring water intake following injection of 10 ng angiotensin II (Sigma-Aldrich) in 1 μ l 0.9% saline in water-replete rats. Briefly, intra-cerebroventricular (icv) injections in the third ventricle of 1 μ l angiotensin II solution were carried out using internal injectors (Plastics One) placed inside the implanted guide cannula. The solution was delivered slowly with the injector left in place for 60 s before removal. Only those rats that consumed at least 5 ml of water within 1 h of injection were included in the subsequent studies. Rats recovered for an additional week before receiving any BDNF injection. Those rats that passed angiotensin II-induced drinking test were allowed to recover for another week and were used in further tests.

Body weights and daily food intake were monitored and blood samples from tail bleeding were collected for estradiol measurement before testing effects of central BDNF administration on feeding. Similar hormone treatment and pair-feeding paradigms as *Experiment 2* were used in this experiment. When tested for central BDNF anorectic effect, OVX-Oil PF rats were allowed to eat *ad libitum* for two days, on BDNF injection day and the following day, before pair-feeding was resumed. Rats from other groups were *ad libitum* fed.

BDNF administration and food intake: BDNF injections were carried out a day after subcutaneous injection of estradiol or oil, thus Female Sham-Oil rats were tested during endogenous estrus and OVX-E2 rats were tested on the days that modeled estrus (Asarian and Geary 2002). Each rat received three injections with a counterbalanced design, 0 μ g (saline), 0.1 μ g, or 0.3 μ g BDNF, with a one-week interval among tests to allow for clearance of BDNF from the CNS and for normal feeding to be reestablished. We selected these doses of BDNF because 0.1 μ g BDNF is a subthreshold dose and 0.3 μ g BDNF has been shown to be minimally effective to suppress feeding in male rats in a pilot study (unpublished results).

On these test days, food was removed from the animal cages 4 h prior to lights-off. Rats then received icv injections of 0 (saline), 0.1 μ g, or 0.3 μ g BDNF (Millipore, Temecula, CA) in 1 μ l 0.9% saline vehicle at a rate of 1 μ l / min right before the onset of dark. Food was

returned to the animal cages, and food intake, corrected for spillage, was measured 4 h and 24 h post-injection. Following each test, rats were allowed to recover for one week before the next injections. Hormone replacement (estradiol and oil) and drug treatment (different doses of BDNF) were counterbalanced across treatment.

Statistical analysis

Data were analyzed using Prism Statistical Software 5 (La Jolla, CA). Data were expressed as mean $\pm$ SEM. In *Experiment 1*, an independent two-sample t test was used to analyze average male and female body weights and daily food intake, a one-way repeated-measures ANOVA followed by Tukey's posttest was used to analyze daily food intake and body weight of female rats across an ovarian cycle, and a one-way ANOVA followed by Bonferroni's multiple comparison test compared with Male group was used to analyze plasma estradiol level and *Bdnf* expression in the VMH and in the PVN. In *Experiment 2*, a two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison test compared with Sham-Oil group was used for analysis of daily food intake and body weight, and a one-way ANOVA followed by Bonferroni's multiple comparison test compared with Sham-Oil group was used to analyze plasma estradiol level and *Bdnf* expression in the VMH and in the PVN. Additionally, correlation analysis between plasma estradiol level and *Bdnf* expression was conducted using linear regression, R^2 was calculated to indicate goodness of fit, and F and P values were calculated to indicate significance of correlation (Prism Statistical Software 5). In *Experiment 3*, a one-way ANOVA followed by Tukey's posttest was used to analyze plasma estradiol levels, body weight, and food intake before BDNF central injections, and a two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison test compared with 0 μ g BDNF was used for analysis of food intake. A test with p-value less than 0.05 (*i.e.* $P < 0.05$) was considered statistically significant. *Post hoc* multiple comparison tests were carried out with computing the 95% confidence interval (CI) of the difference between two group means and the test was considered statistically significant if this CI did not include zero.

Results

Experiment 1: *Bdnf* gene expression of intact male and female rats

The average body weights of male ($n = 10$) and female ($n = 40$) rats were 358.48 ± 5.61 g and 244.58 ± 1.09 g, respectively. On average, male and female rats consumed 21.59 ± 1.11 g and 13.71 ± 0.27 g standard rodent chow daily. Thus, age-matched female rats weighed less [$t_{48} = 32.61$, $P < 0.0001$, Cohen's $d = 11.77$] and consumed less amount of food daily [$t_{48} = 10.24$, $P < 0.0001$, Cohen's $d = 3.69$] than male rats. Consistent with previous studies (Asarian and Geary 2002), female rats exhibited cyclic changes in feeding across the ovarian cycle. Daily food intake of female rats was highest between metestrus and diestrus (14.32 ± 0.36 g), declined during proestrus (13.79 ± 0.40 g), reached its nadir between proestrus and estrus right after the estrogen surge (12.70 ± 0.32 g), and then increased between estrus and metestrus (14.03 ± 0.39 g, Fig. 1A) [$F_{(3, 39)} = 5.98$, $P = 0.0008$, partial $\eta^2 = 0.13$]. Daily food intake was significantly decreased between proestrus-estrus compared with metestrus-diestrus periods [$P < 0.05$]. Changes in female body weight across the ovarian cycle showed a trend that mirrored the changes in food intake (Fig. 1B) [$F_{(3, 39)} = 2.74$, $P = 0.05$, partial $\eta^2 = 0.07$], but no significance in any pairwise comparison of the body weight across the ovarian cycle was observed.

Circulating estradiol levels and central *Bdnf* expression of female rats were measured from each of four stages of the ovarian cycle, proestrus, estrus, metestrus and diestrus ($n=10$). Plasma estradiol peaked at proestrus (Fig. 1C). Additionally, the plasma estradiol levels at proestrus (55.70 ± 3.00 pg/ml), estrus (37.60 ± 0.78 pg/ml) and metestrus (30.80 ± 0.93 pg/

ml), but not at diestrus (26.20 ± 0.70 pg/ml), were significantly higher than that of male rats (23.50 ± 0.54 pg/ml, Fig. 1C) [$F_{(4,45)} = 73.46$, $P < 0.0001$, partial $\eta^2 = 0.87$]. The expression of *Bdnf* within the VMH also changed across the ovarian cycle. VMH *Bdnf* mRNA level of estrous rats ($224.89 \pm 21.91\%$), but not rats at other ovarian stages, was significantly higher than that of male rats ($100 \pm 17.81\%$, Fig. 1D) [$F_{(4,45)} = 4.50$, $P = 0.004$, partial $\eta^2 = 0.29$]. Therefore, *Bdnf* expression within the VMH was modulated under normal physiological condition in gonadally intact male rats and in female rats that were cycling normally. Additionally, *Bdnf* expression dynamically changed across the ovarian cycle, and the temporal increase in *Bdnf* expression coincided with the decline in feeding between proestrus and estrus which occurred right after the estradiol peak at proestrus. When all five groups of male and female rats were included in analysis, the correlation between plasma estradiol level and VMH *Bdnf* expression was not significant [$F_{(1,48)} = 3.50$, $P = 0.07$, $R^2 = 0.07$]. However, when only the data of two groups of estrous and diestrous rats were analyzed, plasma estradiol level and VMH *Bdnf* expression were significantly correlated [$F_{(1,18)} = 8.05$, $P = 0.01$, $R^2 = 0.31$].

Bdnf mRNA levels in the PVN were not significantly different among female rats at different stages of the ovarian cycle and male rats (Fig. 1E) [$F_{(4,45)} = 0.71$, $P = 0.59$, partial $\eta^2 = 0.06$]. Additionally, there was no significant correlation between plasma estradiol level and PVN *Bdnf* expression of all male and female rats [$F_{(1,48)} = 0.01$, $P = 0.91$, $R^2 = 0.0003$] or of estrous and diestrous female rats [$F_{(1,18)} = 1.71$, $P = 0.21$, $R^2 = 0.09$].

Experiment 2: *Bdnf* gene expression of OVX rats with cyclic estradiol replacement

Sham-Oil and OVX-E2 rats displayed normal ovarian cycles. In contrast ovarian cycles of oil-treated OVX rats (OVX-Oil rats and OVX-Oil PF rats) displayed anestrus. Consistent with previous reports (Wade and Gray 1979; Asarian and Geary 2002), OVX-Oil rats eliminated the cyclic decrease in food intake associated with the ovarian cycle and induced a marked and lasting increase in food intake beginning on the fourth day after surgery compared with Sham-Oil rats [$P < 0.05$] (Fig. 2A). In contrast, OVX-E2 rats displayed only temporal hyperphagia compared with Sham-Oil rats between day 3 and day 6 post-surgically [$P < 0.05$]. From cycle 2 through cycle 5 of estradiol treatment, food intake of OVX-E2 rats varied cyclically with frequencies and amplitudes similar to those of Sham-Oil rats (Fig. 2A). *Post hoc* tests revealed main effects of hormone treatment [$F_{(2,27)} = 60.75$, $P < 0.0001$, partial $\eta^2 = 0.34$] and testing days [$F_{(22,594)} = 8.52$, $P < 0.0001$, partial $\eta^2 = 0.11$]. There was a significant interaction effect between treatment and testing period for daily food intake [$F_{(44,594)} = 4.134$, $P < 0.0001$, partial $\eta^2 = 0.11$]. All four groups of rats ($n=10$), Sham-Oil, OVX-E2, OVX-Oil, and OVX-Oil PF, had similar pre-surgery body weights on Day 0 (Fig. 2B). OVX induced hyperphagia resulted in a rapid body weight gain for OVX-Oil rats relative to Sham-Oil rats (Fig. 2B). Beginning on the ninth day after surgeries, OVX-Oil rats had significantly greater body weights than Sham-Oil rats [$P < 0.05$], whereas OVX-E2 and OVX-Oil PF rats had similar body weights as Sham-Oil rats (Fig. 2B). *Post hoc* tests revealed main effects of hormone treatment [$F_{(3,36)} = 11.89$, $P < 0.0001$, partial $\eta^2 = 0.28$] and testing days [$F_{(23,828)} = 165.10$, $P < 0.0001$, partial $\eta^2 = 0.30$]. There was a significant interaction effect between treatment and testing period for body weights [$F_{(69,828)} = 16.25$, $P < 0.0001$, partial $\eta^2 = 0.09$].

OVX-Oil and OVX-Oil PF rats had significantly lower plasma estradiol levels (18.2 ± 1.18 pg/ml and 20.2 ± 1.74 pg/ml, respectively) compared with Sham-Oil rats at estrous phase (39.90 ± 1.43 pg/ml, Fig. 2C) [$F_{(3,36)} = 63.66$, $P < 0.0001$, partial $\eta^2 = 0.84$]. Circulating estradiol of OVX-E2 rats (41.00 ± 1.74 pg/ml) was replaced to a similar level as estrous Sham-Oil rats by subcutaneous injections of 2 μ g estradiol once every fourth day (Fig. 2C). Therefore, disruption of ovarian cycling by OVX decreased circulating estradiol level, increased daily food intake, and promoted weight gain. Cyclic regimen of estradiol

replacement at a physiological level, beginning from the second treatment cycle, normalized food intake and body weight to the levels observed in sham operated female rats.

OVX-Oil rats had significantly lower *Bdnf* mRNA levels in the VMH compared with Sham-Oil females ($54.25 \pm 5.74\%$ vs. $100.00 \pm 11.38\%$, Fig. 2D) [$F_{(3,36)} = 6.22$, $P = 0.002$, partial $\eta^2 = 0.34$]. This decrease in *Bdnf* expression induced by OVX was completely restored by cyclic replacement with estradiol (OVX-E2: $107.16 \pm 17.77\%$) but not by pair-feeding (OVX-Oil PF: $57.66 \pm 3.88\%$, Fig. 2D), two groups of rats that consumed same amount of food and had similar average body weights, indicating that estradiol directly, but not secondary to the changes in feeding or body weight, regulates *Bdnf* expression in the VMH. Furthermore, there was a significant correlation between plasma estradiol level and VMH *Bdnf* expression [$F_{(1,38)} = 19.60$, $P < 0.0001$, $R^2 = 0.34$]. In contrast, *Bdnf* mRNA levels in the PVN were comparable among all four groups (Fig 2E) [$F_{(3,36)} = 0.29$, $P = 0.83$, partial $\eta^2 = 0.02$], and there was no significant correlation between plasma estradiol level and PVN *Bdnf* expression [$F_{(1,38)} = 0.005$, $P = 0.95$, $R^2 = 0.0001$]. These data suggested that *Bdnf* gene expression within the VMH, but not the PVN, was regulated in an estradiol-sensitive manner.

Experiment 3: Anorectic effect of central BDNF in male and female rats

Similar to what was observed from vaginal cytology samples in *Experiment 2*, the ovarian cycles of Female Sham-Oil rats were not affected by surgical procedure or drug administration. Following postsurgical recovery and cannula placement verification, the average body weight of Male Sham-Oil rats was greater than all four female groups including Female Sham-Oil, OVX-E2, OVX-Oil, and OVX-Oil PF (Table 1, * $P < 0.0001$). Additionally, *ad libitum* fed OVX-Oil rats had greater body weights than the other three groups of female rats (Table 1, # $P < 0.0001$) [$F_{(4,33)} = 72.06$, partial $\eta^2 = 0.90$]. Daily food intake of male rats was also significantly greater than that of all four groups of female rats (Table 1, * $P < 0.0001$) [$F_{(4,33)} = 17.33$, partial $\eta^2 = 0.68$] and was not significantly different among the female groups. Consistent with the results from *Experiment 1* and *2*, plasma estradiol levels of Female Sham-Oil and OVX-E2 rats were higher than those of Male Sham-Oil rats (Table 1, * $P < 0.0001$) and both groups of oil-treated OVX rats (OVX-Oil and OVX-Oil PF) (Table 1, # $P < 0.0001$) [$F_{(4,33)} = 20.49$, partial $\eta^2 = 0.71$].

There was no significant difference in 4 h or 24 h food intake among four female groups following 0 μ g BDNF injection. Specifically, 4 h and 24 h food intake after 0 μ g BDNF injection were 3.53 ± 0.36 g and 14.72 ± 1.10 g for Female Sham-Oil rats, 3.09 ± 0.31 g and 14.99 ± 0.82 g for OVX-E2 rats, 4.28 ± 0.94 g and 18.44 ± 0.96 g for OVX-Oil rats, and 4.39 ± 0.84 g and 17.91 ± 0.99 g for OVX-Oil PF rats. In contrast, Male Sham-Oil rats consumed more food 4 h and 24 h after 0 μ g BDNF injection (6.17 ± 0.46 g and 21.50 ± 1.68 g, respectively) than did Female Sham-Oil and OVX-E2 rats ($P < 0.05$) and consumed more food 4 h after injection than did OVX-Oil rats ($P < 0.05$).

BDNF at dose of 0.1 μ g, a sub-threshold dose in male rats, significantly decreased 4 h and 24 h food intake in Female Sham-Oil rats and OVX-E2 rats ($P < 0.05$), but failed to influence food intake in OVX-Oil or OVX-Oil PF rats (Figs 3A and 3B). A larger dose of BDNF (0.3 μ g) decreased 4 h and 24 h food intake in all groups including oil-treated male and OVX rats. *Post hoc* tests revealed significant main effects of hormone treatment on the 4 h food intake [$F_{(4,33)} = 8.76$, $P < 0.001$, partial $\eta^2 = 0.30$] and 24 h food intake [$F_{(4,33)} = 14.69$, $P < 0.0001$, partial $\eta^2 = 0.34$], and significant main effects of BDNF doses on the 4 h food intake [$F_{(2,66)} = 35.78$, $P < 0.001$, partial $\eta^2 = 0.20$] and 24 h food intake [$F_{(2,66)} = 45.53$, $P < 0.0001$, partial $\eta^2 = 0.26$], while the interaction effect was not significant. Therefore, the effective dose of BDNF tested that caused significant reduction in food intake

was lower in estrous Female Sham-Oil and OVX-E2 rats than in male and OVX-Oil rats, suggesting that estradiol modulates the anorectic effect of BDNF.

Discussion

Estradiol exerts an inhibitory effect on feeding that is well characterized in the rat. For example, the preovulatory increase in estradiol secretion in female rats is associated with a phasic decrease in food intake during estrus (Drewett 1973; Blaustein and Wade 1976), OVX stimulates sustained hyperphagia that is mediated by decline in estradiol secretion (Wade 1975), and physiological regimen of estradiol replacement reverses OVX-promoted hyperphagia (Asarian and Geary 2002). Estradiol does not act alone to inhibit feeding, instead, it enhances the strength of anorectic signals that function to decrease meal size, such as cholecystokinin (Wager-Srdar, Gannon et al. 1987; Butera, Bradway et al. 1993; Geary, Trace et al. 1994; Eckel and Geary 1999; Geary 2001), fenfluramine (Eckel, Rivera et al. 2005; Rivera and Eckel 2005), glucagon (Geary and Asarian 2001), and apolipoprotein A-IV (Shen, Wang et al. 2010); and lessens the strength of orexigenic signals that function to increase meal size such as melanin-concentrating hormone (Messina, Boersma et al. 2006; Santollo and Eckel 2008) and ghrelin (Clegg, Brown et al. 2007).

The primary goal of this study was to determine whether *Bdnf* expression and the anorectic effect of BDNF are modulated by estradiol levels. In support of our hypothesis, *Bdnf* gene expression within the VMH, but not within the PVN, was regulated in an estradiol-sensitive manner and the sensitivity to BDNF-induced anorexia was regulated by the estradiol levels. First, VMH *Bdnf* expression was dynamically regulated across the ovarian cycle with a temporal elevation at estrous phase following estradiol peak, which coincided with the decline in feeding at this phase of the ovarian cycle, and there was significant correlation between circulating estradiol level and VMH *Bdnf* expression in estrous and diestrous female rats (*Experiment 1*), suggesting that change of the VMH *Bdnf* expression across the ovarian cycle may play a role in the cyclic changes in food intake of female rats. The estrous and diestrous rats were chosen for analysis of correlation between endogenous estradiol level and *Bdnf* expression, because the estradiol level of estrous rats was comparable to those of Sham-Oil rats sacrificed on estrous day and OVX-E2 rats sacrificed the next day after cyclic estradiol injection; and the estradiol level of diestrous rats was comparable to those of OVX-Oil and OVX-Oil PF rats (Fig. 1C and 2C). Second, consistent with previous reports (Wade and Gray 1979; Asarian and Geary 2002), OVX promoted hyperphagia and eliminated the cyclic decrease in food intake associated with the ovarian cycle, resulting in a rapid increase in body weight and a decrease in *Bdnf* expression relative to sham-operated females. A cyclic regimen of estradiol treatment that mimics the changes in estradiol secretion in cycling female rats (Asarian and Geary 2002) reversed all of these alterations. In addition, there was a significant correlation between plasma estradiol and VMH *Bdnf* expression among sham-operated rats, and OVX rats with or without estradiol replacement (*Experiment 2*). These data raise the possibility that estrus-associated decrease in food intake, which is well characterized in female rats, may be mediated, at least in part, by an increase in endogenous BDNF signaling in the VMH. Third, a lower dose (0.1 µg) of BDNF suppressed food intake in estrous rats and OVX rats with cyclic estradiol replacement, but not in male rats or oil-treated OVX rats, suggesting that the minimally effective dose of BDNF to induce anorexia was lower in rats with higher circulating estradiol levels within physiological range. These findings provide the first evidence that physiological levels of estradiol, either endogenous or replaced cyclically, increased *Bdnf* expression within the VMH and sensitivity to BDNF-induced reduction in feeding.

Estradiol exerts a delayed effect in feeding due to the genomic effect via its nuclear estrogen receptors. For example, cycling female rodents have suppressed feeding during estrus, a

stage after estradiol peaks at proestrus (Tarttelin and Gorski 1971; Asarian and Geary 2002; Asarian and Geary 2006). Additionally, the plasma estradiol level is the highest on the first day of estradiol injection, but suppression in food intake begins on the second day of estradiol treatment (Asarian and Geary 2002). Interestingly, a similar delay between the peak of plasma estradiol level at proestrus and elevated VMH *Bdnf* expression at estrus was observed in the current study (Fig 1C and 1D), suggesting that estradiol affects central *Bdnf* expression possibly due to the genomic effects of estradiol via its nuclear receptors ER α and/or ER β .

Bdnf expression within the PVN was not correlated with plasma estradiol levels in either cycling female rats or in OVX rats with or without cyclic estradiol replacement. Different from the VMH, the PVN with predominant expression of ER β and little expression of ER α (Shughrue, Komm et al. 1996; Shughrue, Lane et al. 1997; Österlund, G.J.M. Kuiper et al. 1998; Shughrue and Merchenthaler 2001) is not critically involved in estrogen's anorectic action, and there is no colocalization of ER and BDNF in the PVN. Therefore, different from the VMH, the PVN is not likely a site for the involvement of BDNF in estrogen-regulated anorectic action.

Although *Bdnf* expression is concentrated in the VMH, there is a widespread expression of its receptor *TrkB*, including the VMH, the dorsomedial nucleus, and the arcuate nucleus (Kernie, Liebl et al. 2000), indicating that the central anorectic effect of BDNF is not limited at the VMH. To our knowledge, the current study is the first one that investigated the interaction between estradiol and BDNF in the regulation of feeding. The first step is to test whether the central anorectic effect of BDNF is regulated by estradiol levels. BDNF was injected into the third ventricle, and consequently acted on many hypothalamic sites where *TrkB* is distributed. The data from current study suggest that the central anorectic effect of BDNF is regulated by circulating estradiol levels. Whether BDNF's anorectic effect within a specific region is regulated by estradiol levels remains to be defined. In future, BDNF will be infused into specific hypothalamic regions to study specific sites for the interaction between estradiol and BDNF.

A pair-feeding method was used in *Experiment 2* and *Experiment 3* so that a group of OVX-Oil rats and OVX-E2 rats were fed an identical amount of food. This technique was used to determine whether differences in *Bdnf* expression and BDNF-induced anorexia between oil- and estradiol-treated OVX rats remain once their body weights are similar. By controlling the amount of energy intake, OVX-Oil PF rats had similar body weight to those of Sham-Oil and OVX-E2 rats (Fig 2B and Table 1); however, the plasma estradiol level of OVX-Oil PF rats were still lowered compared with those of Sham-Oil and OVX-E2 rats (Fig 2C and Table 1). Interestingly, VMH *Bdnf* expression (Fig 2D) and BDNF-induced feeding suppression (Fig 3) were similarly down-regulated in OVX-Oil PF and OVX-Oil rats compared with Sham-Oil and OVX-E2 rats. Thus, lower BDNF expression in the VMH is the result of exposure to HFD rather than the resulting weight gain. Therefore, these data indicate that estradiol, instead of difference in body weight, plays an important role in the regulation of expression and anorectic effect of BDNF.

It has been shown that estradiol binds to estrogen receptors, most notably estrogen receptor alpha (ER α) in the hypothalamus (Geary, Asarian et al. 2001; Roesch 2006), and interacts with multiple neuropeptides and neurotransmitters to suppress feeding (Asarian and Geary 2006). VMH plays an essential role in feeding suppression, as illustrated by the hyperphagia triggered by lesions (Anand and Brobeck 1951; Penicaud, Larue-Achagiotis et al. 1983) and the anorexia triggered by stimulation (Valenstein, Cox et al. 1968) to this region in rodents. Compelling evidence supports that the VMH is one of the important hypothalamic sites for estradiol's anorectic action. For example, administration of estradiol into the VMH reduces

food intake (Butera and Czaja 1984; Palmer and Gray 1986), estradiol elevates the number of immunoreactive cells for c-fos, a marker for neuronal activity, within the VMH (Insel 1990), ER α is abundantly expressed in the VMH (Shughrue, Lane et al. 1997; Chakraborty, Hof et al. 2003), and inhibition of ER α by RNA interference in the VMH results in severe obesity and metabolic syndrome (Musatov, Chen et al. 2007). All these available studies suggest the importance of the VMH in estradiol-mediated inhibition in feeding. Hypothalamic *Bdnf* mRNA levels are highest in the VMH (Xu, Goulding et al. 2003; Unger, Calderon et al. 2007), the same site where abundant ER α is located, suggesting that BDNF producing neurons may serve as possible targets for estradiol's actions.

How these two signals, estradiol and BDNF, interact with each other to decrease feeding needs further investigation. One possible mechanism is that estradiol modulates *Bdnf* gene expression and increases BDNF signaling within the VMH. In support of this hypothesis, physiological doses of estradiol increased *Bdnf* mRNA levels in the VMH of gonad-intact rats (*Experiment 1*) and estradiol-treated OVX rats (*Experiment 2*). Thus, estradiol may act on a population of cells to up-regulate transcription of the gene that encodes BDNF and, thereby, to increase the anorectic effect of BDNF. Further experiments are needed to investigate the molecular mechanism(s) as to how central *Bdnf* gene expression is regulated by estradiol.

The second possible mechanism is that estradiol and BDNF may act in the same hypothalamic neurons to regulate feeding. Colocalization of BDNF- and ER α -expressing cells within same neurons has been found in the VMH (Blurton-Jones, Kuan et al. 2004), suggesting that estradiol, after coupling with ER α , may act on BDNF neurons locally in the VMH to facilitate BDNF's anorectic effect. This observation of ER α on BDNF-containing VMH neurons provides a morphological substrate for a potential cross-talk between estradiol and BDNF neuronal systems, and provides structural support for investigating the mechanisms through which estrogen regulates BDNF signaling and anorectic activity in the hypothalamus. Such intrahypothalamic interaction between estradiol and BDNF may be a vital component in the BDNF's action.

The third possible mechanism may involve estradiol modulation of the BDNF receptors TrkB, such as number and binding affinity of TrkB. Because TrkB receptors have been localized within multiple brain regions implicated in the control of food intake (Yan, Radeke et al. 1997), it would be important to determine whether cells containing TrkB also contain estrogen receptors and, thereby, TrkB expressing cells would serve as action targets for estradiol. In order to further investigate a causal relationship between estradiol and BDNF in the regulation of feeding, a logical follow-up to the current study is to test whether estradiol's anorectic effect would be attenuated if BDNF's action is blocked. One way to block the action of BDNF is to block its receptor TrkB. Caution needs to be taken for such experiment, however, because BDNF is not the only ligand for TrkB, as TrkB is also activated by neurotrophin (NT)-4, and to a lesser extent, by NT-3 (Schechterson and Bothwell 2010). Thus, blockage of TrkB receptor may also interrupt NT's action on feeding (Tsao, Thomsen et al. 2008). Another way is to study estradiol's effects in mice with reduced *Bdnf* expression (Lyons, Mamounas et al. 1999; Rios, Fan et al. 2001; Xu, Goulding et al. 2003).

The fourth possible mechanism underlying estradiol-BDNF interaction may involve their upstream melanocortin neurons associated with feeding. Estradiol influences synaptology in the arcuate neurons (Naftolin, Mor et al. 1996) and triggers robust increase in excitability of the arcuate proopiomelanocortin (POMC) neurons (Gao, Mezei et al. 2007). *Bdnf*-expressing neurons in the VMH receive direct neuronal projections from POMC neurons and are downstream mediator of arcuate melanocortinergic system (Xu, Goulding et al. 2003). Additionally, following activation of POMC neurons, leptin stimulates *Bdnf* gene

expression and increases BDNF protein levels in the VMH (Komori, Morikawa et al. 2006). Although speculative, it is possible that excitability of melanocortinergeric POMC neurons may be a regulatory target for estradiol to interact with BDNF. Additional studies are necessary to elucidate the mechanism(s) by which estradiol facilitates BDNF to decrease food intake.

In conclusion, the present study demonstrated dynamic change in *Bdnf* expression across the ovarian cycle which coincided with the cyclic change in feeding of normally cycling female rats. Additionally, cyclic estradiol replacement not only increased *Bdnf* expression within the VMH but also enhanced the sensitivity of BDNF anorectic effect in OVX rats. These data collectively suggest that an increased endogenous BDNF signaling in the brain may mediate estradiol-induced inhibitory effect on feeding.

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Abbreviations

BDNF	brain-derived neurotrophic factor
E2	estradiol, 17- β -estradiol-3-benzoate
ERα	estrogen receptor α
OVX	ovariectomy
PF	pair-feeding
PVN	paraventricular nucleus of hypothalamus
VMH	ventromedial nucleus of hypothalamus

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Highlights

- Temporal elevation of *Bdnf* expression coincided with decline in feeding at estrous phase.
- Cyclic estradiol treatment reversed OVX-induced decrease in *Bdnf* expression.
- BDNF suppressed feeding was significantly greater in estrous rats compared with male rats.
- BDNF suppressed feeding was significantly greater in estradiol-treated OVX rats compared with oil-treated OVX rats.

Fig. 1. Cyclic change in daily food intake during female ovarian cycle coincides with changes in circulating estradiol level and *Bdnf* expression of female rats (*Experiment 1*)

Food intake (A) and body weight (B) of female rats were measured at individual phases of the ovarian cycle. Data represent mean $\pm$ SEM ($n = 40$). Y-axis of Figs 1A or 1B does not start from zero to clearly show differences in food intake or body weight among different phases of the ovarian cycle. One-way repeated-measures ANOVA followed by Tukey's posttest indicated that food intake was significantly decreased between proestrus-estrus compared with metestrus-diestrus periods (*, $P < 0.05$; Fig 1A).

Plasma estradiol level (C), and *Bdnf* mRNA levels in the VMH (D) and in the PVN (E) were measured in male rats and female rats across female ovarian cycle. Data represent mean $\pm$ SEM ($n = 10$). One-way ANOVA followed by Bonferroni's multiple comparison test compared with Male group was performed. *, $P < 0.05$.

Fig. 2. Cyclic estradiol replacement normalizes postovariectomy food intake, body weight, circulating estradiol level and VMH *Bdnf* expression (Experiment 2)

Daily food intake (A) and body weight (B) were measured before and after surgeries from sham-operated rats and OVX rats during five consecutive cycles of treatment with estradiol (2 μ g once each fourth day, indicated by arrows) or oil. The paired rats (OVX-Oil PF) were provided with the same amount of food consumed by estradiol-treated OVX (OVX-E2) rats each day. Data represent mean $\pm$ SEM (n = 10). Y-axis of Figs 2A or 2B does not start from zero to clearly show differences in food intake or body weight among different groups. Two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison test

compared with Sham-Oil group was performed. *, $P < 0.05$ OVX-Oil vs. Sham-Oil; #, $P < 0.05$ OVX-E2 vs. Sham-Oil.

Plasma estradiol level (C), and *Bdnf* mRNA levels in the VMH (D) and in the PVN (E) were measured in sham-operated rats at estrous phase and oil- or estradiol- treated OVX rats one day after oil or estradiol injection. Data represent mean $\pm$ SEM ($n = 10$). One-way ANOVA followed by Bonferroni's multiple comparison test compared with Sham-Oil group was performed. *, $P < 0.05$ OVX-Oil or OVX-Oil PF vs. Sham-Oil.

A

B

Fig. 3. Endogenous estradiol of sham-operated female rats or cyclic estradiol replacement of OVX rats increases the anorectic effect of BDNF (*Experiment 3*)
 Food intake was measured at 4 h (A) and 24 h (B) after icv administration of BDNF (0, 0.1, or 0.3 µg). Data represent mean $\pm$ SEM ($n=7-8$). A two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison test compared with 0 µg BDNF was used for analysis of food intake. *, $P < 0.05$ comparing to 0 µg (saline) injection of each group.

Table 1

Body weights, daily food intake and circulating estradiol levels of rats before receiving BDNF injections (*Experiment 3*).

Groups	Body weight (g)	Food intake (g)	Plasma estradiol (pg/ml)
Male Sham-Oil (n = 8)	378.51 ± 10.44 #	22.28 ± 1.22	16.38 ± 1.60 #
Female Sham-Oil (n = 8)	254.30 ± 2.88 *	14.47 ± 0.44 *	35.25 ± 2.09 *
OVX-Oil (n = 8)	292.44 ± 4.70 *#	17.34 ± 0.68 *	17.25 ± 2.43 #
OVX-Oil PF (n = 7)	258.53 ± 5.35 *	Paired to OVX-E2 rats	20.86 ± 3.36 #
OVX-E2 (n = 7)	255.86 ± 5.03 *	15.50 ± 0.96 *	38.29 ± 1.69 *

Male Sham-Oil = male rats Sham surgery and Oil injection;

Female Sham-Oil = female rats Sham surgery and Oil injection;

OVX-Oil = female rats OVX surgery and Oil injection;

OVX-Oil PF = female rats OVX surgery and Oil injection, paired to OVX-E2 rats;

OVX-E2 = female rats OVX surgery and E2 injection.

All data presented as mean ± SEM (n=7–8). One-way ANOVA followed by Tukey's posttest was performed. P-value less than 0.05 (*i.e.* $P < 0.05$) was considered statistically significant.

* indicated significant differences compared with Male Sham-Oil group.

indicated significant differences compared with Female Sham-Oil group.