Discussion
Discussion

Effect of Ovariectomy on FI, WI and BW

Results of the present study suggest that ovariectomy increases FI, WI and BW. Ovariectomy induced increase in FI and WI were transient, lasting less than eight weeks while the BW was elevated and sustained at higher levels throughout monitoring period (75 days) compared to sham operated controls. Our data replicate the findings of many others who have observed similar increments in FI, WI and BW, following ovariectomy (11,12,16,51,52,59).

However, the reasons for the transient increase or decrease in FI and WI and persistent increase or decrease in BW, following ovariectomy and EB treatment are highly controversial. There is some evidence that ovariectomy increases hypothalamic NPY expression (261) and decreases hypothalamic corticotrophin-releasing hormone (CRH) immunoreactivity (262), both of them would promote hyperphagia. It has been suggested that the transient changes in FI coincided with transient changes in NPY concentrations in the paraventricular nucleus of hypothalamus (PVN), whereas sustained effect on BW was found to be associated with leptin insensitivity and decreased spontaneous physical activity, not due to reduced energy expenditure (17), a finding consistent with the earlier observations (52). In contrast, others have reported that the BW loss induced by estrogen may not be due to increase in locomotor activity (57,263) since ethamoxytriphetol (MER-25) did not stimulate voluntary exercise in running wheels in rats, although, it decreased FI and BW. MER-25 mimics estrogen actions but it does not affect running wheel activity in rats (57,263). Although, we have not measured physical activity levels, our animals displayed reduced physical activity following ovariectomy, indicating locomotor activity may in part, played a role in the development of
hyperphagia and obesity. It could be argued that reduced physical activity in rats, following ovariectomy is a mere result of their increased BW, but in one study (17) the BW did not correlate consistently with activity level, indicating that locomotor activity in ovariectomized rats is being controlled by some other mechanisms. Others have reported that high FI may account for the reduced activity (10).

Another possibility is that ovarian hormones might affect BW and adiposity through an action on brown adipose tissue (264-267). However, neither the sympathetic activity nor the thermogenic activity in brown adipose tissue was suppressed after ovariectomy (268), suggesting that either of these two factors account for the BW gain or loss in ovariectomized (OVX) or OVX-EB treated rats. Liver is another metabolically active tissue that contains cytosol estrogen receptors (156) and it may represent a possible ovarian steroid target tissue. It remains to be established whether liver activity is suppressed in rats after ovariectomy.

Ovariectomy model was tested in the subsequent experiments for two reasons. Firstly, to know the site (s) of action of estradiol in the central nervous system (CNS) and secondly, to know its nature of interaction with dopamine receptors that involve in the regulation of ingestive behaviors and body weight. Earlier studies have shown that VMH, NSL and BLA contain estrogen and dopamine receptors (98-103,130). It has been demonstrated that both in ovariectomized and obese Zucker rats there was permanent increase in meal size (MZ) and corresponding decrease in meal number (MN) results in increase in FI and BW. Conversely, EB treatment reverses these changes in the MZ and MN (148). On the other hand, low D2 receptor expression in the hypothalamus was found to be relevant to hyperphagia, associated with large MZ that in turn facilitates in the
development of obesity (90). Dopamine inhibits significantly the firing of neurons in the VMH that signal satiety, in obese than normal rats and D2 receptors mediate this response (170).

Therefore, we postulate that if low dopamine D2 receptor expression is crucial for the development of hyperphagia and obesity as evident from above discussion, then by analogy, it is possible that ovariectomy induced changes in hyperphagia and body weight gain in the present study could be due to down regulation of D2 receptors in VMH, NSL and BLA. D1 receptor down regulation would expect to bring alterations in fluid balance.

**VMH GROUP**

**Effect of intra-VMH injection of EB on FI, WI and BW**

Previous studies have demonstrated that VMH is the major neural site for estrogen mediated effects on feeding, as VMH has a high density of estrogen-binding (98,142), and estrogen sensitive neurons that act on the VMH to reduce FI and BW (15,104). The results of the present study confirm previous findings and there is a dose dependent decrease in FI, WI and BW, following EB injections into VMH in ovariectomized rats, suggesting that VMH plays an important role in mediating anorectic (hypophagia) and weight reducing effects of estrogen.

Present study replaces the implantation (diluted/undiluted) technique with diluted estradiol (daily) injections directly into the nucleus under study. It has been reported that this dilute implants restricts the spread of steroid within the brain and periphery by reducing the amount of hormone in the implants, thereby making the hormone less available for diffusion (143). Earlier studies have shown that pure crystalline (undiluted) implants of estrogen spreads widely throughout hypothalamus and results in cornified
vaginal smears and increased uterine weights (143,144) and these peripheral changes produced by leakage from the central implant sites might account for the observed changes in FI, WI and BW. On the contrary, the presence of cornified vaginal smears was not associated with larger effects on FI, BW or lordosis; therefore, the peripheral changes produced by leakage from the central implant sites were neither necessary nor sufficient to account for the observed behavioral changes (143).

Others have demonstrated that estrogen induced anorexia is due to elevated plasma triglyceride levels (258), thyroxine, and thyroxine in turn could affect locomotor activity (269,270). However, we did not observe any significant changes in vaginal cytology, uterine weights and thyroid hormone concentrations (T3 and T4), total triglyceride and total cholesterol concentrations in the serum (data not shown). Our findings indicate that there was no leakage from nucleus to peripheral circulation and observed effects of estrogen in VMH may not account either for thyroid hormones, plasma lipids or for vaginal cytology and uterine weights. Probably, estradiol might have enhanced the physical activity and contributed for reduction in FI and BW. It has been reported that during estrus in normal rats spontaneous activity greatly increases and FI decreases and consequently loss of BW (9). Further, the transient changes in FI following ovariectomy or EB treatment, coincide with transient changes in NPY concentrations in the paraventricular nucleus of hypothalamus (PVN), whereas sustained effect on BW was found to be associated with leptin insensitivity and decreased spontaneous physical activity, not due to reduced energy expenditure (17). On the other hand, electrolytic lesions in VMH increases FI and displayed persistent reduced activity (9), while unilateral implants of EB in VMH depress FI without affecting locomotor activity (15). Although, we
have not measured physical activity levels, our animals have displayed some restlessness (hyperactivity) during estradiol treatment, in contrast to ovariectomized rats that had shown reduced activity. Therefore, we speculate that reduced FI and increased activity could be reason for the loss of BW when EB was injected into VMH.

Present study demonstrates that EB inhibits WI in VMH. However, the magnitude of decrease in WI was less than FI and BW, following injection of EB into VMH. Previous studies have reported that estrogen inhibits WI (11,12,60). In female rats, water consumption fluctuates in estrous cycle (67), being lowest on the day of estrus (12). Withdrawal of estrogen by ovariectomy abolishes this fluctuation in water consumption (12), indicating that estrogen is an important factor in modifying spontaneous water consumption in female rats. It has been reported that estrogen injection does not significantly inhibit WI in normal rats, it does so in ovariectomized, water deprived and isoproterenol treated rats (66).

Effect of s.c EB before and after VMH lesion on FI, WI and BW

There was a dose dependent inhibition in FI, WI and BW following subcutaneous injection of EB in ovariectomized rats prior to lesion of the VMH. These findings accord with previous studies in that peripheral estrogen is sufficient to prevent the increase in FI and BW in ovariectomized rats (147-149). Due its lipophilic nature, it is possible that EB would cross the blood brain barrier to act on VMH, as discussed above. VMH plays crucial role in the estrogen mediated effects of FI and BW, as it contains maximum number of estrogen receptors. Earlier, it has been argued that estrogen receptors in VMH are not adequate enough to account for EB inhibitory effects and estrogen receptors more than one site in the brain may contribute additively to the estrogenic inhibition of
feeding (145). Further, it has been reported that if the VMH is the crucial site for estrogenic modulation of ingestive behaviors and body weight, then rats with VMH lesions should exhibit smaller increases in FI and WI and gain less BW than non-lesioned controls following ovariectomy. Conversely, they should also show smaller reductions in FI and WI and lose less weight following peripheral treatment with EB (15). In our study, ovariectomized animals have shown significant additive effect on FI and BW without significant change in the WI, in VMH lesioned rats. However, the additional increase in FI and BW with VMH lesions was far less compared to sham controls (Table-14). Previous reports have shown that rats with VMH lesions gained additional weight when given ovariectomies (150,271) and others have reported that ovariectomy did not result in additional weight gain in goldthioglucose treated obese mice (151) or obese VMH rats given ovariectomies gained less than control rats given ovariectomies (272).

On the contrary, in our study, VMH lesioned rats have shown significant but small reductions in FI and lost less BW, following subcutaneous injection of EB. However, the magnitude of inhibition for FI and BW was remarkably less in lesioned rats compared to pre-lesioned rats (Fig.5). In contrast, inhibition of EB on WI was significant and unabated, because VMH lesions had no effect on WI in ovariectomized rats. By and large, our findings are consistent with the hypothesis that VMH contains receptors which monitor estrogen and affect FI, WI and BW regulation. It was evident from earlier studies that neurons in the VMH can directly inhibit feeding when exposed to estrogen (15,104), probably they might influence feeding related behaviors when body weight exceeds a certain level (104). In the absence of VMH receptors, other brain or peripheral loci may monitor estrogen effects and reduce feeding normally, although suppression of BW may
not be to that extent when the VMH receptors were intact. This dissociation between changes in FI and BW in response to estrogen treatment in animals with VMH lesions may occur because estrogen does not produce the usual increase in activity in the lesioned animals (10) or it may result from complex changes in endocrine-metabolic function consequent to the VMH lesions (273-276). Alternatively, it could be argued that ovariectomy resulted in smaller increases in FI and BW in the lesioned animals because the VMH lesions reduced circulating levels of estrogen prior to ovariectomy. This is supported by reports of gonadal atrophy following VMH lesions (277,278), although others did not observe significant changes in ovarian weight resulting from the VMH lesions (272). Therefore, we support the view that estrogen probably causes only a fine tuning of the body weight set point within a restricted range, and the obesity after estrogen withdrawal is mild when compared to with the obesity induced by VMH lesions (47). Therefore, even if estrogen acts directly upon VMH to induce its inhibitory effects (15), there must be some other inhibitory mechanisms that depend upon the integrity of VMH which remain operative in ovariectomized rats to regulate FI and BW, well below the maximal levels (52). However, central blockade of dopamine D2 receptors in VMH were insufficient to affect feeding behaviors and body weight, following subcutaneous injection of EB, as many other receptors and/or areas of the brain that also control these behaviors are intact.
**Effect of intra-VMH injection of dopamine agonists on FI, WI and BW**

Apart from estrogen, yet another important neurotransmitter that influences FI, WI and BW in VMH (159) is DA. DA exerts its inhibitory effects via DA receptors (74). There are several subtypes of DA receptors viz., D1, D2, D3, D4 and D5 (75) and broadly classified as D1-like (D1 and D5) and D2-like (D2, D3 and D4) (76). Neuroimaging studies have revealed that pathological overeating and obesity are associated with significant reduction in D2 receptors in obese individuals than normal individuals (79). However, to our knowledge there are no studies regarding the role of dopamine receptors in VMH involved in the pathophysiology of hyperphagia and weight gain in ovariectomized rats. Therefore, we have investigated whether intra-VMH injections of dopamine and its specific dopamine D1 and D2 (SKF-38393 and Bromocriptine) receptor agonists and antagonists have any role in the regulation of ingestive behaviors and body weight in ovariectomized rats.

The results of the present study demonstrate that intra-VMH injection of dopamine SKF and BC (D1 and D2 receptor agonists) inhibits FI, WI and BW gain in a dose-dependent fashion. The effects of SKF were more pronounced on WI than on FI and BW, in contrast, BC inhibited predominantly FI and BW. Our findings corroborate with previous works in that selective agonists of dopamine D1 (SKF-38393) and D2 (Bromocriptine) decrease FI and BW (69,163,164). Available evidence suggests that intracerebroventricular (163) administration of bromocriptine into VMH reduces BW, insulin resistance, glucose intolerance, hyperinsulinemia, and body fat stores in Syrian hamsters, and reduces elevated levels of NE and 5-HT. (164). Further, it has been reported that obese and diabetic animal models have increased hypothalamic
noradrenergic and serotonergic activities (164,173-176) and reduced brain dopamine synthesis (173), while bromocriptine treatment ameliorated these abnormalities (164). Present data demonstrate that ovariectomy induced hyperphagia and body weight gain were effectively prevented by bromocriptine. Therefore, our findings implicate that D2 receptors in VMH may in part, regulate ingestive behaviors and body weight in ovariectomized rats.

It has been found that the D2 receptors levels in normal rats were quite high in VMH and LHA, compared to D1 receptors (99,100). In vivo, microdialysis study has shown that in the normal rat, the release of dopamine (DA) in the LHA and VMH correlates with meal size (MZ) and postmeal intervals, reflecting the meal number (MN) (168,169). Therefore, the normal function of dopaminergic transmission in VMH is indispensable for feeding and survival (159). Dopamine acting locally within the hypothalamus, acts as a potent inhibitor of feeding in perifornical area, ventromedial hypothalamus and arcuate nucleus (161,162). DA is a potent inhibitor of hypothalamic NPY expression and activity and a stimulator of pro-opiomelanocortin expression. These hypothalamic influences may contribute to DA's ability to reduce food consumption and hyperphagia (161,162). In obese Zucker rats, there was an up-regulation of D1 receptor mRNA in VMH and adenohypophysis (AH) and down-regulation of the same in LHA, whereas D2 receptor mRNA was down regulated in both VMH and LHA but no change in AH, compared to lean rats (90). Others have reported that dopaminergic D1 and D2 receptor mRNA in the hypothalamus were highly expressed in anorectic tumor bearing (TB) rats compared with non-tumor bearing (NTB) free feeding normal rats. These changes were associated with anorexia and the characteristic decrease in MN and
concurrent increase in MZ at the onset of the anorexia. Specifically, the highly expressed D1 receptors in the VMH relate to the increase in MZ, whereas the highly expressed D2 receptors in the LHA and VMH contributed to the anorexia via a decrease in MN (172) suggesting that MZ and MN are regulated independently via different systems (279,280). Thus in the light of above mentioned discussion it appears that in VMH-D2 receptors contribute primarily in the regulation of FI and BW, whereas, D1 receptors involving in the fluid regulation.

On the other hand, in obese Zucker rats, dopamine inhibits significantly the firing of neurons in the VMH that signal satiety, and D2 receptors mediate this response (170). In the same line, D2 receptors might have mediated ovariectomy induced effects on FI and BW. Because our present study demonstrates that ovariectomy induced increase in FI and BW were inhibited by bromocriptine. It has been suggested that though, the basal concentration of dopamine in VMH is not different in Zucker obese and lean rats (281,282), exaggerated VMH DA response to eating occurs in obese Zucker rats (177). In obese Zucker rats, higher concentrations of the feeding related amines (5-HT and DA) are necessary to bring about satiety, because of the low postsynapical D2 expression, where DA acts as a satiety signal via a feedback to the hindbrain feeding centers. Therefore, hyperphagia in obese Zucker rats may be the result of a resistance to the prandially released satiety promoting neurosubstances (177).

**Effect of intra-VMH injection of dopamine antagonists on FI, WI and BW**

Our data show that intra-VMH injections of D1 (SCH-23390) antagonist led to predominant increase WI, but no significant change was observed in FI and BW. Conversely, D2 antagonist (sulpiride) resulted in increase in FI and BW without affecting
However, previous studies have suggested that intra-VMH injection of the D1 receptor antagonist in tumor bearing rats (TB-SCH-23390) led to significant and persistent decrease in FI. D1 receptor blocking via intra VMH SCH-23390 (D1 receptor antagonist) in non-tumor bearing free feeding rats, (NTB-SCH) had no significant effect on FI. The injection of intra-VMH D2 receptor antagonist (sulpiride) significantly stimulated FI in tumor bearing rats relative to their controls i.e., tumor bearing sulpiride control rats (TB-SC). In non-tumor bearing free-feeding rats (NTB-Sul), intra-VMH sulpiride also increased FI significantly compared to baseline intakes. Further, the low D2 receptor expression in VMH induces hyperphagia and FI after sulpiride injection was greater in obese rats but had no effect on lean rats (90), indicating the strong D2 receptor involvement in the regulation of ingestive behaviors and body weight regulation in VMH.

It has been suggested that the D1 receptors stimulate adenyl cyclase activity (76), which in the VMH facilitates prolactin release from the pituitary (283) while, prolactin in turn stimulates FI (284). In contrast, D2 receptors suppress adenyl cyclase activity inhibiting prolactin release (76,285). Therefore, D1 receptors in VMH may be involved in stimulating FI via prolactin release, whereas D2 receptors in VMH may be involved in suppressing FI via inhibiting the prolactin release (172). It has been reported that long-term administration of D2 receptor antagonists increases FI and BW in female rats (26) and it could be either by blocking hypothalamic D2 receptors (22,26,95,286,287) or by decreasing serum estradiol level due to hyperprolactinemia or to a direct effect of sulpiride in hypothalamus (25,95), or by changing insulin sensitivity (288,289). However, sulpiride does not affect FI and BW in male rats (290). In humans, treatment with antipsychotic drugs that block D2 receptors leads to significant weight gain (291,292).
Our present data suggest that in VMH, D1 agonists and antagonists predominantly influence WI on the other hand D2 mainly on FI and BW in ovariectomized rats. Previously, it has been reported that D2 receptors were significantly associated with their body mass index (BMI) in obese humans but not in control individuals and the D2 receptors may not be involved in modulating body weight per se, but rather may regulate compulsion to eat in the pathological eater (79). Further, it has been suggested that down regulation of D2 receptors may represent a common pathogenic mechanism contributing to obesity (90) and addiction to various types of drugs including cocaine (91) alcohol (92) and opiates (93). Although, we did not assess the dopamine receptor expression and DA concentrations in VMH, we speculate that low D2 receptor expression in VMH may in part, account for the pathophysiology of FI and BW in ovariectomized rats. Because, ovariectomy and sulpiride induced hyperphagia and weight gain was effectively prevented by BC and EB, respectively.

Effect of EB-DA interaction in VMH on FI, WI and BW

It has been reported that the direct actions of estradiol on central dopaminergic system may be critical to control the FI and BW (3). Further, noradrenaline, dopamine and serotonin influence ingestive behaviors and body weight via VMH (42,293). In addition, monoamines, NE promotes and 5-HT (5-hydroxytryptophan) inhibits feeding behavior, while dopamine modulates these responses (3). Estrogens have also been found to inhibit FI, presumably by inhibiting the activity of dopamine beta-hydroxylase, thereby causing an increased DA (294) and a decrease in norepinephrine levels (89). When the concentration of estrogen is elevated, dopamine is unable to convert to noradrenaline and it causes decrease in FI (3). Therefore, the present study hypothesizes
that dopamine may be a plausible neurotransmitter that facilitates this response through its specific receptors that involve in the control of these measures as described above. If dopamine in VMH facilitates estrogenic suppression of ingestive behaviors and body weight, estrogen in ovariectomized rats, should prevent the stimulatory effects of dopamine receptor antagonists on FI, WI and BW compared to controls. To establish this we have injected SCH and Sulpiride (D1 specific antagonist and D2 specific antagonist) with estradiol benzoate (EB).

Our present study demonstrates that the EB could prevent Sulpiride induced increase in FI and BW, whereas, SCH stimulated WI in VMH. These findings suggest, in VMH estrogen induced change in FI and BW is mediated primarily by D2 receptors and hypodipsic effect of estrogen is mediated by D1 receptors. It has been reported that ovariectomy increases meal size permanently (149) and estradiol seems to have a tonic inhibitory effect on the light phase meal size and phasic effect on the dark phase meal size and meal number, but no significant effect on the light-phase meal number (295). Reports suggest that, increase in meal size and decrease in meal number, and low dopaminergic D2 receptor mRNA expression in the VMH was found to be relevant to hyperphagia (90). Estrogen stimulates a significant increase in D2 receptors in the striatum (296), and in vivo administration of the estrogen increases the number of DA receptors indicating an important role for estrogens in maintaining the normal function of DA in the CNS (297). Further, it has been reported that pathological overeating and obesity are associated with significant reduction in D2 receptors in the striatum of obese humans (79) as well as rats (94) compared to controls. Contrarily, estrogen may act via stimulation of the catecholestrogens and these catecholestrogens seems to be
biochemical link between estrogens and catecholamines in the central nervous system to modulate daily rhythm in feeding (295).

Taken together, the above findings support our hypothesis that dopamine plays an important role in the regulation of feeding related behaviors and body weight in ovariectomized rats, mediating these effects predominantly by D2 receptors in VMH.

NSL GROUP

*Effect of intra-NSL injection of EB on FI, WI and BW*

NSL seems to be a major extra-hypothalamic region involved in the regulation of energy balance in the female rat (180) and the septal-hippocampal complex has been implicated for various psychoneuroendocrine processes including regulation of feeding behavior and body weight (105,180,182). Both autoradiographic and biochemical studies in female rats have demonstrated the presence of estrogen receptors (98,130) and distribution of estrogen receptor beta (ER-β) immunoreactivity (133) in NSL. Our findings are first to demonstrate that direct injection of EB into NSL in female rats, following ovariectomy resulted in a dose dependent decrease in FI, WI and BW. The magnitude of inhibition for FI and BW was more than WI indicating the role for NSL in energy balance. Although, the mechanisms that govern energy balance in NSL largely not known, it could be due to enhanced activity of estrogen sensitive neurons in the NSL to inhibit these behaviors. Because previous studies have reported that estrogen sensitivity has been attenuated in lateral septum with kainic acid lesions (105,182), and changes in FI preceded the increase in BW with septal damage, they were due to modifications in the estrogenic control of feeding but not in the alteration in pituitary or metabolic function (180). Like in VMH, we did not find any significant effect on estrus smears, uterine
weights, serum thyroid hormone and lipid profile concentrations, indicating that there was no leakage from nucleus to peripheral circulation and observed effects of estrogen in NSL may not account for these peripheral responses. EB, probably increased estrogen sensitive neurons in NSL and this may in part, resulted in inhibition of FI, WI and BW.

**Effect of s.c EB before and after lesion in NSL on FI, WI and BW**

Like in VMH group, there was a dose dependent inhibition in FI, WI and BW, following subcutaneous injection of EB in ovariectomized rats before lesion. Because, there is a possibility that peripherally administered EB can act at multiple sites in the brain to bring about extra inhibitory effect on these behaviors. Therefore, to establish the exact role of the estrogen in modulating energy balance through NSL, we have made lesions in NSL following ovariectomy, and later subcutaneous injections were given in lesioned rats.

Data obtained from the present study shows that there was significant increase in FI, WI and BW in ovariectomized animals, following NSL lesions but the additional increase was considerably less compared to sham operated controls (Table-27). Conversely, lesioned rats displayed smaller reductions in FI, WI, and lost less BW, following subcutaneous injection of EB compared to pre-lesioned rats (Fig.7). However, our findings only implicate that estrogen affects FI, WI and BW to some extent are mediated via NSL, as the observed changes were not as great as that of VMH. Previous works have demonstrated that lesion of the NSL with neurotoxins kainic acid and domoic acid produce a significant increase in FI and BW in female rats but not in male rats (141,180-182) and NSL is sexually dimorphic with respect to its role in neural control of BW (181). These effects could be due to attenuation of the estrogen anorexic effects on FI and BW, as evidenced by earlier studies and they were not due to decrease in the
percent days of vaginal smears (105,180,182). It has been reported that the effects of kainic acid lesions on energy balance were directly proportional to the extent and location of the NSL damage. However, in earlier studies it has been shown that the single injection of EB (6µg) reduced FI and BW of the electrolytic septal lesions significantly, whereas the FI and BW of the kainic acid septal lesions were not significantly altered (105).

In the present study, increase in WI following lesion of NSL was highly significant compared to FI and comparable to BW (Table-27), implicating the importance of the NSL in mediating drinking behavior. Earlier workers have reported that kainic acid induced hyperdipsia was transient (184,298) and this hyperdipsia does not reflect increased prandial requirements due to overeating, as WI during 24 h food deprivation caused only slightly and significantly higher than that in controls (183). The peculiar variability in the effectiveness of septal lesions with respect to WI appears to be related to their effects on cholinergic pathways. Lesions that produce hyperdipsia result in significant decreases in brain acetylcholine, lesions that fail to affect WI apparently do not (299). Further, central injection of sulpiride into NSL could not affect FI, WI and BW induced by subcutaneous injection of EB in non-lesioned ovariectomized rats, as many other centers of the brain that control these behaviors are intact.

Effect of intra-NSL injection of dopamine agonists and antagonists on FI, WI and BW

The dose-dependent inhibition of FI and BW was observed following injections of BC into NSL, while injection of SKF resulted in significant inhibition of FI and BW and that occurred only at higher dose. These findings suggest that D2 receptors in NSL primarily
involve in the regulation of Fl and BW than D1 receptors. Further the magnitude of inhibition was greater for BW than Fl following bromocriptine injection, indicating that D2 receptors mediated hypophagia is transient, and may be to stabilize the reduction in BW. However, neither D1 nor D2 agonist had any significant effect on WI, suggesting that D1 and D2 receptors in NSL may not take part in the regulation of WI. Present findings on WI are in good agreement with the previous reports from our laboratory that dopamine had no role in the control of WI (190). However, NSL has efferent connections with specific hypothalamic nuclei involved in the control of energy balance (300,301) and limitations in our study (please see below) did not permit us to make definite statements whether or not NSL exerts its control over ingestive behaviors and body weight, directly or indirectly through its hypothalamic connections. Therefore, further studies are required to assess the role of other neurotransmitters in NSL in the control of ingestive behaviors and body weight, because septum contains variety of neurotransmitters (191-194) that might influence these measures directly or indirectly.

Effect of EB-DA interaction in NSL on Fl, WI and BW

Administration of EB into NSL considerably prevented both SCH and SP induced increase on Fl and BW. However, EB effects were more potent in preventing SP induced changes than SCH. The effect on WI was not significant because, neither SP nor SCH had any role in the regulation of WI. Our data suggest that dopamine has some role in the EB mediated actions on Fl and BW in NSL. Although, we do not have direct evidence from the present study, it is possible to speculate that the effects of EB on these measures were mediated via D2 receptors in NSL, by increasing density of dopamine.
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receptors particularly, D2 receptors and thereby contributing to the amelioration of ovariectomy induced alterations in ingestive behaviors and body weight in NSL.

BLA GROUP

Effect of intra-BLA injection of EB on FI, WI and BW

Only the highest dose (4μg) of EB injected into BLA has resulted in significant inhibition on FI, WI and BW. However, the degree of inhibition was small, in comparison to VMH and NSL. This may partly reflect the presence of few labeled estrogen neurons in BLA (98,130), compared to VMH and NSL. Further, BLA has few estrogen receptors compared to other parts of the amygdala (302). However, observed effects of EB on FI, WI and BW may be due to the fact that presence of high estrogen receptor-beta (ER-β) immunoreactivity in female rats and higher ER-β protein and mRNA expression in the lateral amygdaloid nucleus (134). Moreover, there is a wide distribution of ER-β mRNA in basolateral amygdala (246). We speculate that in female rats, ER-β presumably to some extent played a role in the modulation of ingestive behaviors and body weight in BLA, though it merits further research to prove this possibility. However, previous works have suggested that in female rats the EB implanted into the cortical amygdala can reduce FI without requiring the integrity of pathways in the VMH-Arcuate region (106). Others have reported that medial amygdala may monitor estradiol levels in the blood and cerebrospinal fluid and may send information about the stage of the estrous cycle to modulate gonadotropin release and feeding in female rats (250).

Effect of s.c EB before and after lesion in BLA on FI, WI and BW

Like in VMH and NSL groups, there was a dose dependent inhibition in FI, WI and BW following subcutaneous injection of EB in ovariectomized rats before lesion of the
BLA. Data obtained from the present study shows that there was an additional increase in FI, WI and BW in ovariectomized animals following BLA lesions, but this was extremely small compared to sham operated controls (Table-39). Conversely, ovariectomized rats with lesions have shown significant but smaller reductions in FI, WI, and lost less BW, following subcutaneous injection of EB compared to rats with intact nucleus (Fig.9).

Although, the role of BLA in the regulation of ingestive behaviors is highly complex and controversial, the present observations implicate that BLA in some way participates in estrogen regulated ingestive behaviors, albeit, to a smaller extent when compared to VMH and NSL. It has been reported that VMH with its numerous glucose receptors may monitor hunger, whereas amygdala with its major input from olfactory bulb may control feeding behavior (228). Others have reported that after functional disruption of BLA (235,236) resulted in conditioned taste aversion (CTA) but not the other amygdaloid nuclei (235,237,238) and also neophobia (234). Therefore the observed changes may not entirely be due to estrogenic effects. CTA and neophobia could have influenced in reduced intakes and thereby loss of body weight. However, central injection of sulpiride into BLA could not significantly affect FI, WI and BW induced by subcutaneous injection of EB in non-lesioned ovariectomized rats, as other amygdaloid nuclei that regulate these behaviors are intact.

**Effect of intra-BLA injection of dopamine agonists and antagonists on FI, WI and BW**

Injection of SKF into BLA significantly decreased WI without significant inhibition of FI and BW. Conversely, BC significantly inhibited FI and BW without affecting WI. However, the degree of inhibition induced by dopamine agonists, especially on FI and
BW injected into BLA was less compared to inhibition that obtained in VMH and NSL group. The effect of SKF in BLA on WI was comparable to VMH.

Our present data suggest that D1 receptors in BLA may participate in the regulation of WI, while D2 receptors in the control of FI and BW though magnitude of effect was far less compared to other two nuclei. Present findings indicate that dopamine in BLA might have affected FI, WI and BW, possibly via its influence on prefrontal cortex (mPFC) and nucleus accumbens (NAC), because, DA terminals in both these regions of the rat play an important role in the attribution of incentive value to sensory properties of biologically relevant stimuli and facilitate appropriate behavioral responses (251).

**Effect of EB-DA interaction in BLA on FI, WI and BW**

In BLA, EB has prevented SCH induced increase in WI and SP induced increase in FI and BW. However, only highest doses (4μg) of EB had significant effects on SCH and SP induced changes in FI, WI and BW, unlike VMH and NSL where EB with low doses could exert significant effects. From, these findings it is conceivable that role for both dopamine and estrogen in the control of ingestive behaviors and BW is minimal compared to VMH and NSL. This may be due to presence of small number of both estrogen and dopamine receptors in BLA, as mentioned in the literature. Nevertheless, D1 receptors exhibited predominant effect on WI while D2 receptors mainly on FI and BW. We suspect that EB might have inhibited dopamine efflux in mPFC and NAC and it would have resulted in decrease of incentive value for adjunct behaviors (251). Alternatively, EB in BLA may send inhibitory signals to the hypothalamus to suppress hypothalamic feeding center thereby contributing for the decrease in FI and BW. However, given the paucity of literature, absence of, direct measurements of
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neurotransmitters and their immunoreactivity, prevent us to explain the mechanisms by which the BLA exerts its inhibitory effects on these behaviors. Therefore, further studies are warranted to define the role of BLA and mechanisms by which estrogen orchestrates these behaviors in ovariectomized rats.

General Discussion

In intact female rats, food intake (FI), water intake (WI) and body weight (BW) fluctuate with the estrous cycle i.e., low during estrus than diestrus. Therefore, the intact rat oscillates about a steady state of energy balance, losing weight during vaginal proestrus when estrogen levels are high and regaining it during diestrus when estrogen levels are low (9-12). The results of the present study suggest that ovariectomy induced increase in FI and WI were transient, whereas the increase in BW was persistent. Conversely, EB treatment effectively reduced FI and WI transiently and reduction in BW was sustained. These results are in good agreement with many others findings who have observed similar effects on FI, WI and BW (11,12,16,51,52,59), following ovariectomy and EB treatment.

On the whole, present data demonstrate that EB induced inhibition on FI and BW was more pronounced in VMH compared to other two nuclei (i.e., NSL and BLA). These findings imply that VMH is the predominant site of action of EB for the regulation of FI and BW and this may be due to presence abundant estrogen receptors and estrogen sensitive neurons in the VMH compared to NSL and BLA (98,130). Therefore, we do not agree with the contention of earlier workers that estrogen receptors are insufficient in VMH to account for inhibitory effect on these parameters (145). Further, the magnitude of inhibition on FI and BW was highly significant, following subcutaneous administration of
EB in OVX-rats with intact nuclei, compared to OVX + lesioned rats and this effect was quite evident for VMH in comparison to NSL and BLA. These findings further support our hypothesis that EB influences these measures predominantly via VMH and followed by NSL and minimal effects were seen in BLA. It may be due to the fact that VMH contains maximum number of estrogen sensitive neurons and receptors than NSL and BLA as reported earlier. However, the degree of EB induced inhibition on WI comparable in VMH and NSL (Fig.10). Rats with NSL lesions exhibited maximum increase in WI compared to VMH and BLA (Fig.15) and this was further evident following s.c injection of EB resulted in small decrease in WI with NSL lesions comrade to rats with VMH and BLA lesions (Fig.16). Previous studies have reported that septal lesions that induce hyperdipsia decrease acetylcholine levels in the brain (299). From our study, it is difficult to explain whether EB mediated WI is due to its effects on cholinergic neurotransmission in NSL.

Subcutaneous EB treatment did not show any significant effect on vaginal cytology, uterine weights and concentrations of serum thyroid hormones and lipids, indicating that there was no leakage from central to peripheral circulation and observed effects may predominantly due to central rather than peripheral actions of EB. Moreover, the 10μg dose injected in the present study was too small to have metabolic effects. Previous studies have reported that 125μg dose was very small to have metabolic effects (297). However, the effect of subcutaneous EB (peripheral administration) was equally potent compared to central injections in mediating these behaviors because due to its (EB) lipophilic nature peripherally administered drug could cross the blood brain barrier to affect VMH, NSL and BLA. Therefore, after the lesion the effect EB on FI, WI and BW
was reduced in all the nuclei because lesions might have destroyed estrogen sensitive neurons.

The biochemical mechanisms involved in the effects of both ovariectomy and EB substitution on FI, WI and BW are unclear at present. In recent years, considerable attention has been devoted to define the underlying role of various neurotransmitters or neuromodulators in the control of estrogen induced energy balance. Previous studies have highlighted the importance of many peptides like leptin (18), NPY (17), CCK (19) and glucagons (20) mediate FI and BW induced by estradiol in ovariectomized (OVX) rats and estrogen modulates WI in ovariectomized rats via the peptide hormone, angiotensin-II, (21). However, it has been reported that the direct actions of estradiol on central dopaminergic system may be critical to control the FI and BW (3). Another possibility may be direct modulation of EB in the daily rhythm of other neurotransmitters such as dopamine, norepinephrine (303) and serotonin (304). It has been suggested that norepinephrine promotes and serotonin terminates eating while dopamine modulates eating responses (3). It has also been demonstrated that estrogen alters D2 receptor expression (305) and functional activity (306) in striatum and nucleus accumbens. Earlier, central dopaminergic neurons have been implicated in the control of FI (68) and stimulation of dopamine receptors reduces FI (307) and both D1 and D2 receptors seem to have this effect (307).

Based on the above evidences, our present findings tempt us to postulate that changes induced by both ovariectomy and EB replacement, may be mediated via dopamine in VMH, NSL and BLA. The strong evidence for this hypothesis comes from our experiments 2-5 in all the nuclei, in that we have demonstrated that ovariectomy
induced increase in FI, WI and BW was suppressed by both bromocriptine and SKF. On the contrary, sulpiride and SCH induced increase in FI, WI and BW was effectively prevented by EB. Both these effects were observed predominantly in VMH compared to NSL and BLA. Presence of high levels of D2 receptors in VMH (99,100) compared to NSL (188) and BLA (103) may, in part, explain why the effect of D2 receptors was predominant in VMH.

Further, it has been suggested that down regulation of D2 receptors may represent a common pathogenic mechanism contributing to obesity (90) and addiction to various types of drugs including cocaine (91) alcohol (92) and opiates (93). Further, it has been reported that pathological overeating and obesity are associated with significant reduction in D2 receptors in the striatum of obese humans (79) as well as rats (94) compared to controls. On the other hand, sulpiride (D2 receptor antagonist) induced body weight gain and obesity in females rats totally prevented by D2 agonist bromocriptine (26,95) or estradiol (25) or tamoxifen (95) administration, which acts as estradiol with respect to FI and BW (96). It has also been reported that bromocriptine treatment significantly reduced body fat stores in post menopausal women (97). Low serum estradiol levels have been observed in antipsychotic treated women with mental disorders (96) and healthy premenopausal women during sulpiride administration (308).

Overall, the above discussion provides some credence to our present hypothesis that changes induced by both ovariectomy and EB replacement may be mediated via dopamine in VMH, NSL and BLA. Further, down regulation of D2 receptors might have contributed for increase in FI, WI and BW, following ovariectomy. Estradiol and bromocriptine (D2 receptor agonist) replacement would expect to increase the D2
receptor densities in VMH, NSL and BLA to correct the abnormalities induced by ovariectomy.

It is uncertain from the present study as to how estradiol and dopamine interact to effect on FI, WI and BW in ovariectomized rats. It has been proposed that permanent increase in meal size (MZ) and corresponding decrease in meal number (MN) results in increase in FI and BW in ovariectomized rats and EB treatment reverses these changes in the MZ and MN (148). Interestingly, obese Zucker rats also display a similar feeding pattern consisting of large MZ and small MN, and decreased D2 receptor expression in the hypothalamus. Moreover, as reported in the literature, the low D2 receptor expression was found to be relevant to hyperphagia associated with large MZ that in turn facilitates in the development of obesity (90). Further it has been shown that sulpiride (D2 receptor antagonist) injected into VMH, facilitated the aggravation of already low D2 receptor expression, causing animal to have larger MZ. Therefore, we postulate that if low dopamine D2 receptor expression is crucial for the development of hyperphagia and obesity, then by analogy, it is possible that ovariectomy induced changes in hyperphagia and body weight gain in the present study could also be due to down regulation of D2 receptors in VMH, NSL and BLA. D1 receptor down regulation would expect to bring alterations in fluid balance. Another plausible mechanism, by which estradiol and dopamine ameliorate ovariectomy induced abnormalities, is physical or locomotor activity. Previous studies have shown that ovariectomy in rats (17) and women (309) undergoing transition to menopause decreases spontaneous physical activity. Estradiol replacement reverses this abnormality in rats and exercise has been found to increase dopamine release (310) and raises D2 receptors (311) in animal models. In addition,
locomotor activity following administrating of D2 agonist varies with sex, females exhibiting a higher response than males (312).

We presume that estrogenic regulation of D2 receptor expression and/or function may be an important neurochemical mechanism by which estrogen ameliorates altered ingestive behaviors and body weight in ovariectomized rats. However, in the present study, predominant effect was found in VMH compared to other two nuclei, because as reported earlier, VMH contains high levels of both estrogen and dopamine receptors.

At present we do not know what molecular mechanisms account for the functional interaction between estradiol and dopamine receptors to affect the ingestive behaviors and body weight in ovariectomized rats. However, earlier works have shown that estradiol can affect both pre- and post-synaptic elements of dopaminergic neurons (313). Further, it has been suggested that D2/D3 receptor function involves a genomic mechanism (305) and estrogen fails to compete directly with dopamine and with D2 agonists for the D2 receptor binding site (314), ruling out direct steroid interactions with binding sites on D2 receptors. Direct modulation of D2 isoform expression also reported (315). D2 receptor is expressed as D2L and D2S isoforms and estrogen induces the expression of D2L splice variant suggesting a mechanism by which estrogen may change the efficiency of D2 receptor-G protein (guanosine-binding proteins) coupling. It has been observed that D2/D3 stimulated G protein activation in cingulate cortex was lower in ovariectomized females than in those with estrogen (305). On the other hand, it has been reported that centrally, estrogen may either act genomically by direct binding to the nucleus, followed by chain of biochemical events, involving DNA-dependent RNA synthesis or via stimulation of membrane receptors via the catecholestrogens (149).
Catecholestrogens are synthesized in many tissues including the hypothalamus and are potent inhibitors of the enzyme catechol-o-methyl transferase and tyrosine hydroxylase. They are unique because of the steroid structure they can act similar to estrogens, and because of the catechol structure they can act similar to and compete with catecholamines (316-318). Interestingly, it has also been suggested that the catecholestrogens may be a biochemical link between estrogens and catecholamines in the CNS, changes that may modulate the daily rhythm of feeding (295). Future studies must explore whether dopamine receptor biosynthesis and/or gene expression is influenced by estradiol to establish the functional interaction between estradiol and dopamine in mediating ingestive behaviors and body weight in VMH, NSL and BLA.