

Effect of subcutaneous injection of estradiol on feeding and drinking behaviors and body weight in basolateral amygdaloid lesioned rats

Bodepudi N Rao¹, Gopal K Pal², Pal Pravati²

¹Department of Physiology, Shri Sathya Sai Medical College and Research Institute, Ammapettai, Kancheepuram (Dist), Tamil Nadu; ²Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, INDIA-605006

KEY WORDS

Estradiol
Feeding
Drinking
Body weight
BLA

ABSTRACT

Background: Estradiol is known to inhibit food intake (FI), water intake (WI) and body weight (BW) across the species including women and it is most evident in rats. Ovariectomy in rats and menopause in women produce hyperphagia and obesity. Estradiol substitution in ovariectomized (OVX) rats and hormone replacement in women reverses these changes suggesting that lack of estradiol causes eating related disorders. However, the neurobiological target/s for estradiol mediating effects remains largely unknown. While lesions of basolateral amygdala (BLA) also produce hyperphagia, polydipsia and obesity in female rats suggesting BLA normally inhibits these behaviors. **Purpose:** Since ovariectomy is a useful model to study postmenopausal obesity in women, we have investigated the role of BLA in ovariectomy induced ingestive behaviors. **Methods:** Ovariectomy and stereotaxic lesions in experimental group (n = 6) whereas sham operations in control group (n = 6) were carried out in female rats. Estradiol was injected subcutaneously (s.c) before and after lesions in experimental group and vehicle was injected in control group. **Results:** Data from the present study shows that there was an additional increase in FI, WI and BW in OVX animals following BLA lesions, but this additive effect was small compared to sham operated controls. Conversely, OVX rats with lesions have shown small but significant reductions in FI, WI, and lost less BW, following s.c injection of estradiol compared to rats with intact BLA. **Conclusion:** These findings suggest that ovariectomy and estradiol induced changes on ingestive behaviors and body weight are partly mediated via BLA.

Corresponding Author:

Bodepudi N Rao, PhD

Tel : +91-9176050362

E-mail : bodepudinarasimharao@gmail.com

doi : 10.5214/ans.0972.7531.200404

Introduction

Feeding and drinking behaviors and body weight fluctuate across mammalian species^{1,2} including women³ throughout their reproductive cycle⁴ with the changes in the concentration of estrogen. Phase related changes in FI, WI and BW^{2,5} are quite evident in rats. After ovariectomy plasma estrogen decreases to a negligible level⁶ and results in increase in FI, WI and BW. While estrogen substitution either centrally⁷ or peripherally,⁸ reverses these effects. Like OVX rats, women also display increase in body weight and adiposity after menopause,⁹ while estrogen replacement therapy reduces these changes.¹⁰ Thus, lack of estrogen in OVX rats and in menopausal women uniquely predisposes them to develop obesity and related disorders. Previous studies in rats have shown that estrogen acts in the specific areas of the brain viz., ventromedial hypothalamus,¹¹ paraventricular nucleus¹² to mediate its anorectic actions.

However, in the present study we have investigated whether BLA has any role in mediating estradiol actions on feeding and drinking behaviors in OVX rats. We have chosen this particular brain region because for decades amygdala is known to influence the ingestive behaviors and the discovery of Klüver-Bucy syndrome provided ample evidence that amygdaloid nuclei affect FI, WI and BW¹³ and lesion studies with BLA produced inconsistent results.¹⁴⁻¹⁶ Further, amygdaloid lesion-induced hyperphagia and obesity are much more prominent in female rats¹⁷ suggesting that there is a sex difference in hyperphagia and weight gain in rats with amygdaloid lesions. Whether similar sex differences are seen in drinking behavior with BLA lesions in rats is not known. Estrogen receptor- β (ER- β) immunoreactivity and ER- β protein and mRNA expression was higher in BLA and is greater in females than in males¹⁸⁻²⁰ but no con-

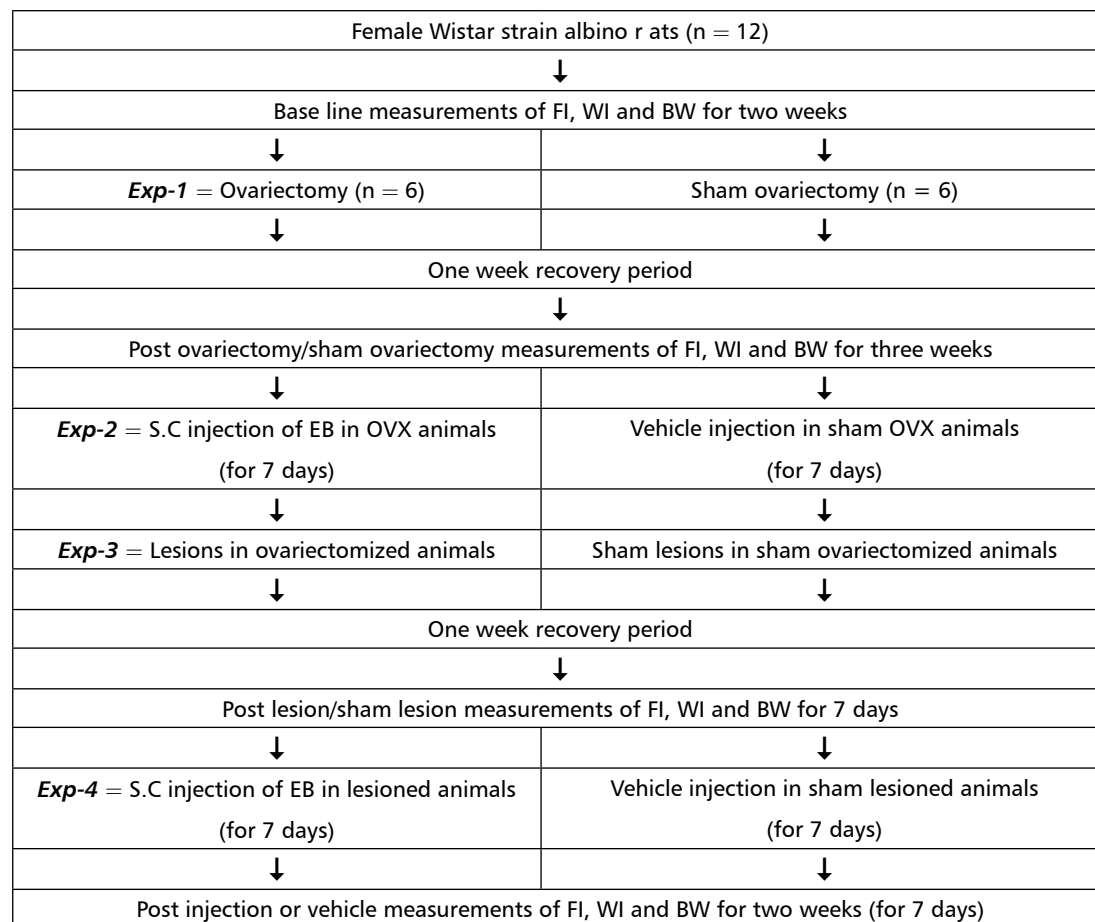
sensus as to which receptors i.e. ER- β or ER- α mediate estrogen actions.²¹ It was found that amygdala also takes up estradiol and affects feeding and hyperphagic rats with amygdaloid lesions are similar in some respects (e.g., lack of finickiness) to ovariectomized rats.²² However, the weight gain produced by amygdaloid lesions and ovariectomy were additive.¹⁷ On the contrary, estradiol benzoate (EB) implanted into the cortical amygdala can reduce food intake,²³ medial amygdala modulate feeding in female rats²⁴ but not the BLA. Therefore, the present study investigates whether bilateral electrical lesions of BLA can produce an additive effect on feeding, drinking behaviors and on BW in OVX rats and we also examined whether s.c injection of estradiol induced decrease in FI, WI and BW gain are mediated via BLA.

Methods

Animals

A total number of institute bred 12 adult, age matched, healthy, female albino rats of Wistar strain weighing 190–240 g at the time of arrival were served for different experimental procedures. All the experimental protocols were performed with the approval of the Institutional Research Council and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), JIPMER, Puducherry, India. The rats were housed individually in cleaned plastic cages at a temperature of 28 to 35°C and maintained throughout the experiments on a 12:12 h light-dark cycle. Animals were fed rat pellets and fresh tap water available *ad libitum* and they were habituated to the cages for two weeks before baseline measurements were made. All the animals were simply handled everyday to minimize stress before and during the experimental procedures.

Study design



Baseline measurements of FI, WI and BW

After adaptation of rats to our laboratory conditions, 24-h food intake (FI) and water intake (WI) and body weight (BW) were measured for 2 weeks between 8.00 am and 10.00 am. The average values on these measures were taken as the pre-ovariectomy (before the removal of ovaries) base lines. For WI, the tap water at room temperature was provided in a calibrated glass cylinder with a sprout and measured to the nearest 0.5 ml. The food was given in the form of standard rodent food pellets. The food was placed in the space for food provided in the cage. FI and BW were measured to the nearest 0.1g using electronic weighing machine (Citizen, MP 500; Japan). Food spillage was collected and subtracted from the intake. Further, vaginal smears were examined daily in all the rats to monitor ovarian cycle and all rats with intact ovaries shown the consistent 4-day estrous cycles.

Surgical procedures and experiments

After the establishment of baseline values for FI, WI and BW, following surgical procedures and experiments were been carried out.

Ovariectomy

This experiment was carried out to investigate whether ovariectomy increases FI, WI and BW compared to sham operated

animals. The experimental rats were subjected to bilateral ovariectomy through a single lower midline abdominal incision, using sterile surgical technique, under xylazine (7 mg/kg, i.p) and ketamine hydrochloride (100 mg/kg, i.p) anesthesia. In experimental rats (n = 6), the right and left ovaries were identified at the junction between the fallopian tube and uterine horn by identifying periovarian fat and removed by a single cut. Immediately after surgery the abdominal wall was sutured using sterile catgut. Control rats (n = 6) received sham ovariectomy in which the ovaries were exposed but left intact. Vaginal smears were collected from all the ovariectomized rats to ensure the success of surgery and no evidence of residual ovarian activity was found. Post ovariectomy FI, WI, and BW were measured for 3 weeks.

Subcutaneous injection of EB in OVX animals/Sesame oil in Sham OVX animals

β-Estradiol-3-Benzoate (EB) was obtained from Sigma Company (Saint Louis, M.O, U.S.A) and the drug was dissolved in sesame oil (vehicle). This experiment was designed to assess whether the BLA has any role in the estradiol induced inhibition on FI, WI and BW. If so, then rats with intact BLA should exhibit maximum decline in FI, WI and BW following subcutaneous injection of EB. The effect of subcutaneous injection of 10 µg EB was

examined for 7 days on 24-h FI, WI and BW in OVX rats and during this period the sham OVX rats received equal volume of vehicle.

Stereotaxic surgery

The aim of this experiment was to investigate whether ovariectomized rats gain additional increase in FI, WI and BW following BLA lesions compared to sham-operated controls. After taking post-ovariectomy FI, WI and BW measurements, the same animals were fixed on a stereotaxic apparatus (INCO, Ambala, India) for producing deep electrolytic lesions in BLA using Konig and Klippel rat stereotaxic atlas.²⁵ Stereotaxic surgery for lesions was performed aseptically under xylazine (7 mg/kg, i.p) and ketamine hydrochloride (100 mg/kg, i.p) anesthesia using the following coordinates from lambda: antero-posterior-3.4 mm, lateral to the mid saggital suture-4.0 mm, dorsoventral-8.2 mm. Bilateral lesions were made in experimental group (n = 6) using lesion maker (INCO, Ambala, India) by passing anodal current between uninsulated tip (0.5 mm) of an insulated stainless steel electrode and a rectal cathode. The anodal current 2 mA for 20s was passed. Animals with sham lesions (n = 6) had holes drilled in the skull at the same coordinates, and electrodes were lowered to a depth 1.0 mm above the target site, but no current was passed. After surgery, animals were placed in single cages and allowed one week for complete recovery. After recovery from the surgery, the effect of post-lesion basal values for FI, WI and BW were made for 7 days and compared with sham-operated control values.

Subcutaneous injection of EB in lesioned animals/sesame oil in sham lesioned animals

This experiment was designed to assess whether the BLA has any role in the estrogen induced inhibition on FI, WI and BW. If so, then rats with BLA lesions should exhibit minimal decline in FI, WI and lose less BW following subcutaneous injection of EB. The effect of subcutaneous injection of 10 µg EB was examined for 7 days on 24-h FI, WI and BW in lesioned rats and during this period the sham lesioned rats received equal volume of vehicle.

Histology

At the end of experiment, all the rats with brain and lesions were sacrificed with an overdose of pentobarbital (100 mg/kg, Nembutal) and were perfused intracardially with 0.9% saline followed by 10% formal-saline solutions. The brains were removed rapidly and post fixed in 10% neutral-buffered formalin for another 48 hours followed by dehydration and embedding in paraffin. Serial 50 µm coronal sections were cut through BLA, on a microtome (ERMA, Japan). Further, these sections were stained with haematoxylin and eosin and examined under light microscope to verify the location and extent of lesions. Reconstruction diagrams of serial brain sections (Fig. 1) were made to show the extent of lesions in the BLA with the aid of micro-projector with reference to the plates of the Konig and Klippel rat stereotaxic atlas.

Statistical analysis

For data analysis, all values are expressed as means ± SEM and percentage of change relative to controls. Differences between means were compared by Student's *t* test and differences among means were evaluated by one way ANOVA (analysis of variance), using In Stat (GraphPad, version 3.05, USA) software.

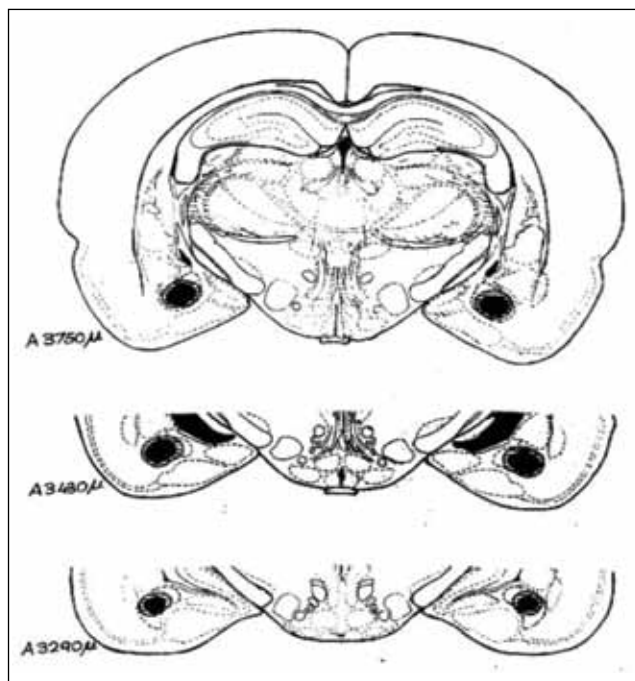


Fig. 1: Reconstruction diagrams of serial coronal brain sections showing the extent of lesions in the BLA with reference to the plates of the Konig and Klippel rat stereotaxic atlas.

Post-hoc test was performed by Tukey Kramer multiple comparison test. The difference was considered statistically significant if probability of chance was less than 0.05 ($p < 0.05$).

Results

Baseline measurements of FI, WI and BW

There was no difference in baseline (pre-ovariectomy) food intake (FI), water intake (WI) and body weight (BW) in both experimental and control animals (Table-1).

Ovariectomy

Following bilateral ovariectomy, there was a significant increase in FI, WI and BW gain in all the experimental rats (Table-2). However, the magnitude of increase in BW was greater ($p < 0.001$) and persistent compared to FI ($p < 0.01$) and WI ($p < 0.05$). The FI reached significance on day 5, and it was maximum on day 8 and stabilized thereafter (with minor fluctuations) through day 21. FI started declining over a period of five days and remained slightly higher to the pre-ovariectomy level and to the control values. Though, WI displayed similar pattern to that of FI, the decline was little earlier than FI i.e., WI started declining on day 17. However, the extent to which the WI increased was less than FI. BW reached significance on day 8 and highest increase was observed on day 12. The gain in BW reached plateau through 21 days of monitoring period and remained approximately 14% above the sham operated controls.

Subcutaneous (s.c.) injections of EB prior to lesion

There was a significant inhibition ($p < 0.001$) in FI, WI and BW when EB (10 mg) was injected subcutaneously for 7 days

Table 1: Pre-ovariectomy basal food intake (FI), water intake (WI) and body weight (BW) of experimental (that were ovariectomized after basal recordings) and control (that were sham operated after basal recordings) rats (n = 6 in each group)

	FI (g)	WI (ml)	BW (g)
Control	12.56 ± 0.50	20.80 ± 0.60	210.40 ± 3.40
Experimental	12.74 ± 0.84	20.92 ± 0.76	211.10 ± 3.20

Values are mean ± SEM of two weeks period prior to ovariectomy or sham-operation. *p* value for FI is 0.8576; for WI is 0.9038 and for BW is 0.8838 and they were not statistically significant.

Table 2: The effect of bilateral ovariectomy (OVX) on food intake (FI), water intake (WI) and body weight (BW) in experimental rats (n = 6) compared to control rats (n = 6) that were sham operated

	FI (g)	WI (ml)	BW (g)
Control	13.63 ± 0.78	21.27 ± 0.86	216.35 ± 3.72
Experimental	18.02 ± 0.92**	24.39 ± 0.90*	246.90 ± 4.56***

Values are mean ± SEM of changes of 14 days. Asterisks indicate values which are statistically significant from the controls. **P*<0.05; ***P*<0.01; ****P*<0.001.

Note: The percentage increase in FI, WI and BW in experimental rats following OVX (compared with values of control rats) was 32.20%, 14.66% and 14.12% respectively.

Table 3: Effect of s.c EB (10 µg) for 7 days on food intake (FI), water intake (WI) and body weight (BW) in OVX-experimental rats (n = 6). Sham operated control rats (n = 6) received equal volume of vehicle

	FI (g)	WI (ml)	BW (g)
Control	17.56 ± 0.68	25.46 ± 0.63	245.30 ± 3.60
Experimental	13.24 ± 0.41*	20.88 ± 0.70*	217.10 ± 3.15*

The values are mean ± SEM; Asterisks indicate values which are statistically significant from the controls. **P*<0.001

Note: The mean% change in FI, WI and BW, following 7 days injection of s.c EB (10 µg) in experimental rats (compared with control rats) was -24.60 ± 0.36, -17.98 ± 0.42 and -11.49 ± 0.56, respectively. Symbol minus (-) indicates the % of inhibition.

(Table-3) in experimental OVX rats compared to vehicle received sham-operated controls.

Electrolytic lesions

Bilateral BLA lesions induced significant increase in FI (*p*<0.05), WI (*p*<0.05) and BW (*p*<0.05) in ovariectomized rats compared to sham lesioned rats that received no current (Table-4).

S.C injections of EB following lesion of BLA

As shown in Table-5, subcutaneous injection of EB (10 µg) for 7 days in BLA lesioned rats, exhibited significant reductions in FI (*p*<0.01), WI (*p*<0.01) and BW (*p*<0.05) compared to post-lesion basal values. However, the magnitude of inhibition was less in lesioned rats, though it was statistically significant, com-

Table 4: Effect of bilateral electrolytic lesion of BLA on food intake (FI), water intake (WI) and body weight (BW) in experimental rats (n = 6). Control rats (n = 6) were sham lesioned

	FI (g)	WI (ml)	BW (g)
Control	17.90 ± 0.80	25.14 ± 0.70	244.80 ± 3.78
Experimental	20.15 ± 0.45*	27.28 ± 0.52*	256.40 ± 3.52*

The values are mean ± SEM of seven days recording following surgery. Asterisks indicate values which are statistically significant from the controls. **P*<0.05

Note: The mean % change in FI, WI and BW following bilateral lesions of BLA of experimental rats (compared with control rats) was +12.56 ± 0.46%, +8.51 ± 0.45% and +4.73 ± 0.52% respectively. Symbol plus (+) indicates the % gain.

Table 5: Effect of s.c EB (10 µg) for 7 days on FI, WI and BW in BLA-lesioned rats (post-lesion EB) of experimental group (compared with their own post-lesion basal). (n = 6)

	FI (g)	WI (ml)	BW (g)
Post-lesion basal	20.15 ± 0.45	27.28 ± 0.52	252.80 ± 3.90
Post-lesion EB	17.40 ± 0.62**	24.18 ± 0.61**	236.60 ± 3.50*

The values are mean ± SEM; Asterisks indicate values which are statistically significant from the controls. **P*<0.05; ***P*<0.01

Note: The mean % change in FI, WI, and BW following subcutaneous injections of EB after lesion of BLA was -13.64 ± 0.42%, -11.36 ± 0.54% and -6.40 ± 0.38% respectively. Symbol minus (-) indicates the % of inhibition.

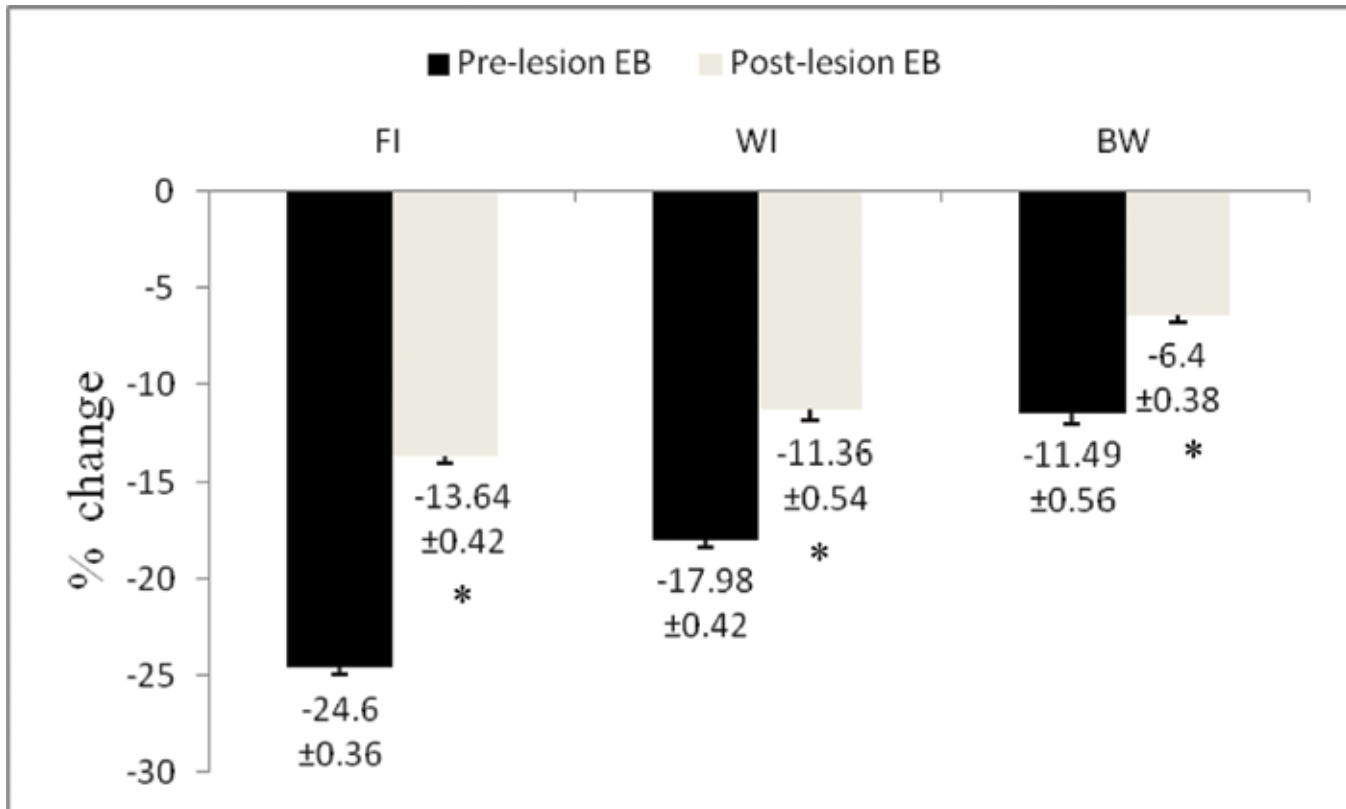
pared to the non-lesioned rats, following administration of subcutaneous EB (Fig. 2).

Discussion

The present study demonstrates that ovariectomy increases FI, WI and BW in rats whereas the s.c injection of EB reverses these changes (Table 2 & 3) and our findings are consistent with previous studies.^{21,26} Since BLA normally inhibits these behaviors,²⁷ peripheral injection of EB could potentiate the BLA satiety effects prior to lesion. This effect of EB would have been mediated via estrogen receptors beta (ERβ) that are found in BLA¹⁸⁻²⁰ and they are known to be involved in the anorectic effects of estradiol in OVX rats.²⁸ There is a possibility that peripherally administered EB can act at multiple sites in the brain including BLA because of its lipophilic nature and estradiol can cross the blood brain barrier to bring about additional inhibitory effect on these behaviors. Therefore, to establish the exact role of BLA on estradiol mediated effects, we have made lesions in BLA following ovariectomy, and later subcutaneous EB given to the lesioned rats.

Bilateral lesions of BLA caused an additional increase in FI, WI and BW in OVX animals (Table-4) compared to sham lesioned animals. Conversely, s.c injection of EB in BLA lesioned rats demonstrated small but significant decrease in FI, WI and BW compared to sham lesion animals (Table-5). However, the percentage of inhibition for these measures was less in post lesion animals compared to prelesion, following s.c injection

Fig. 2: Difference in mean % change in FI, WI and BW in experimental rats (n = 6) induced by s.c EB (10 µg) injected before and after the lesion of BLA.



The values are mean ± SEM; Asterisk indicate values which are statistically significant from the controls. * $P < 0.0001$. Symbol minus (–) indicates the % of inhibition.

Before lesion (Pre-lesion EB) value is the % change in experimental rats, following s.c. EB injection compared with their own OVX-basal. After lesion (Post-lesion EB) is the post-lesion % change induced by s.c. EB.

of EB (Fig. 1). Small additive effect after the lesion and reductions in FI, WI and BW after s.c injection of EB in OVX animals suggests the fact that BLA is also one of the sites in the brain that mediates the EB satiety actions, if not, the only neural target.

Of late, several studies have advocated that estradiol promotes satiety via variety of neuropeptides in the brain. Research has shown that in the brain estradiol either can attenuate orexiogenic effects of neuropeptide Y,^{29,30} ghrelin³¹ or enhance the anorexigenic effects of leptin,^{29,32} cholecystinin,³³ and brain derived neurotropic factor.³⁴ Estradiol also affects the angiotensin-II³⁵ regulation of drinking behavior. It has been reported that both estradiol^{5,36} and dopamine^{37,38} regulate food intake and body weight by moderating both meal size and meal number. There is evidence that neuropeptide Y and dopamine interact antagonistically in the brain in the control of food intake.³⁹ However, our own work suggests that dopamine injected into the ventromedial hypothalamus (VMH) potentiates the estradiol suppression of food intake and body weight.^{40,41} In the light of present findings, we cannot rule out the possible interaction between estradiol and dopamine with its various receptors in the brain including BLA in regulating FI, WI and BW in female rats.

Conclusions

In our present study, we have measured daily FI, along with WI and BW. However, daily food intake (FI), as a function of time, is the product of meal size (MZ) and meal number (MN) [FI = MZ × MN] that constitutes a feeding pattern, while MZ and MN are differentially affected by different manipulations. Since both ovariectomy increases MZ and decreases MN, while EB and treatment reverse this feeding pattern, future studies should include feeding pattern which is a better index to elucidate the neural controls of ingestive behavior, rather than cumulative daily FI. We had earlier demonstrated that dopamine could potentiate estradiol actions on feeding related behaviors in OVX rats in VMH. Therefore, we should also address the interaction between dopamine and its receptor subtypes with estradiol in the brain including BLA on feeding related behaviors and such efforts may provide insights into the pathophysiology of over eating and related disorders in humans. Despite these limitations our findings presented here suggest that estradiol actions on feeding and drinking behaviors and on body weight are mediated partly via BLA in female rats. Further research studies are warranted to assess the application of findings of the present study in the management of eating disorders and obesity

Acknowledgements

We thank Mrs. Selvam Sri Ramachandra Raj and Mrs. Bharati Balakumar at JIPMER for their technical assistance. The data presented in this manuscript is a part of PhD thesis of Rao B.N under the guidance of Pal GK.

This article complies with International Committee of Medical Journal Editor's uniform requirements for manuscript.

Competing interests: None, Source of funding: None

Received Date : 28 September 2013; Revised Date : 25 November 2013;

Accepted Date : 11 December 2013

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