Original Article

Effect of gender on food intake, adiposity and immunological responses following lesion of ventromedial hypothalamus in albino Wistar rats

Venugopal Lalitha, Gopal Krushna Pal, Subash Chandra Parija[1], Pravati Pal, Murugaiyan Sathishbabu[2], Jagadeeswaran Indumathy

Departments of Physiology and [1]Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, [2]Department of Biochemistry, Mahatma Gandhi Medical College and Research Institute, Puducherry, India

Abstract

Background and Aim: The present study was conducted to assess the gender difference in the role of ventromedial hypothalamus (VMH) on the regulation of food intake (FI), body weight (BW), and immunological responses in albino Wistar rats.

Methods: A total of 24 albino Wistar rats were taken for the study and were divided equally into two groups: VMH Group and control Group for VMH lesion, with six male and six female rats in each group. In the experimental group, bilateral electrolytic lesion of the respective nuclei was performed by stereotaxy and postlesion parameters were recorded. In the control group, sham lesion was made. Male-female difference in each parameter was determined.

Results: Following VMH lesion, FI increased significantly in both the sexes ($P < 0.001$) but the percentage increase in FI was more in female rats. Though both male and female rats showed increase in their BW, in males the increase was significant ($P < 0.001$). CD4 (Cluster of differentiation 4) concentration decreased significantly in male rats ($P < 0.001$). Albumin levels decreased significantly ($P < 0.01$) in both male and female rats, following VMH lesion.

Conclusion: The above-mentioned findings suggest that VMH is an important center for satiety and adiposity in rat models and has differential influence on control of FI and BW gain in male and female rats. The impact of VMH on immunity is stimulatory and the system is more developed in males.

Key words: Body weight, food intake, immunity, ventromedial hypothalamus

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INTRODUCTION

Neurophysiological control of immunity is based on the link of hypothalamus and limbic system with sympathetic outflow.[1] Sympathetic fibers innervate immune organs such as thymus, bone marrow, spleen, and lymph nodes and profoundly influence immunological responses.[2] The food intake (FI) and body weight (BW) are known to be influenced by the hypothalamic areas like ventromedial hypothalamus (VMH), lateral hypothalamus, and arcuate nucleus.[3,4] Peripheral signals such as adiposity and calorie intake are integrated by various nuclei within the hypothalamus.[4] Hence, these signals regulate the important pathways within the central nervous system that are involved in control of FI and energy expenditure.[4] Hence, among these brain areas, VMH is the major hypothalamic area that is directly linked to the regulation of FI and BW in animal models.[1] Lesion of VMH produces obesity and some degree of immunomodulation.[2] Obesity *per se* has also been documented to produce immunosuppression.[5] However, the exact nature of change and mechanisms of alteration in immunity in experimentally-induced obesity following neural lesion are not known.

Lesion of VMH leads to alteration in sympathetic activity.[6] Sympathovagal balance has been strongly implicated

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Address for correspondence: Dr. Lalitha Venugopal, Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry - 605 006, India. E-mail: lalitha.jipmer2010@gmail.com

in the regulation of adiposity and there is report of gender difference in sympathovagal modulation of energy homeostasis.[1] However, the exact nature of sympathetic-modulated immunological changes and the gender difference in immunomodulation by these brain areas have not been assessed in these studies. Therefore, in the present study we planned to evaluate the gender difference in FI, adiposity, and immunological responses, following lesion of VMH in albino rats of Wistar strain.

MATERIALS AND METHODS

Animals

After the approval of research council and animal ethics committee of JIPMER, a total of 24 (12 males and 12 females) institute-bred healthy adult albino rats of Wistar strain weighing between 150 and 250 g were obtained for the study. The rats were housed in individual plastic cages with wire lids in the animal research laboratory, department of Physiology, JIPMER. A layer of husk was spread on the floor of the cages. 12 h light–dark cycle was maintained. Standard rodent chow and fresh tap water was available *ad libitum*. Rats were allowed to habituate in individual cages for 10 days before basal measurements were taken.

Basal FI and BW recordings

After 10 days of habituation, 40 g of standard rodent chow and 100 mL of fresh tap water *ad libitum* was provided every day. Daily FI and BW were measured for 1 week to determine the mean 24 h basal recordings.

Groups

Animals were divided randomly into two following groups:

- VMH Group (VMH-lesion made in the VMH bilaterally) (6 males and 6 females)
- Control Group for VMH lesion (sham-lesion of VMH) (6 males and 6 females).

The sample size in each group was 12 (6 males, 6 females). In the VMH group, lesion was made bilaterally in the respective nuclei and weight-matched male and female control animals for VMH group were also included, for which sham lesions were made.

Procedures

Anesthesia

Because the depth of anesthesia required for different procedures was different, the anesthetic agent used was different for different procedures. As light anesthesia was required for blood collection, ether was used as anesthetic agent. For lesion making, inj. Ketamine (0.25 mL/250 g BW) was injected intraperitoneally. For sacrificing the animal, double the dose of ketamine was injected intraperitoneally as described by Dev *et al*.[7]

Blood collection

Approximately, 1.5-2 mL of blood was collected by jugular venous puncture for obtaining the basal immunological values after 7 days of basal readings of BW and FI. For estimation of postlesion immunological parameters, 5 mL of blood was collected with the help of a syringe and needle by puncturing the left ventricle (cardiac puncture) during sacrifice of the animal before fixation of brain.

Electrolytic nuclear lesion

For making lesion, the stereotaxic procedure was performed as described by Pal *et al*.[8] Bilateral electrolytic lesions of VMH were made by introducing electrodes into the nuclei on both sides according to the following coordinates (anterior: 0.45 cm, lateral: ±0.05 cm, vertical: 0.82 cm) obtained from the stereotaxic atlas for rat brain by Konig and Klippel [Figure 2][9] and allowing the anodal current of 0.5 mA to pass through the electrode. In animals undergoing sham lesions, all the above-mentioned steps were followed except that no current was passed.

Parameters

Physical parameters

BW: It was measured in grams every alternate day with an electronic weighing machine for the entire period (4 weeks) of the study.

FI: FI was measured in grams daily with an electronic weighing machine.

After blood collection and lesion/sham lesion procedure, the animals were allowed to recover from the stress of the intervention for a period of 10 days during which FI and BW were not measured.

Immunological parameters

Immunological parameters namely total leukocyte count (TLC) (cells/mm^3 of blood), lymphocyte count (LC) (%), cluster of differentiation 4 (CD4) concentration (pg/mL), cluster of differentiation 8 (CD8) concentration (ng/mL), serum albumin (g/dL), serum globulin (g/dL), Albumin-Globulin ratio (A-G ratio), liver weight-BW (LW-BW) ratio, spleen weight-BW (SW-BW) ratio, serum immunoglobulin M (Ig M) (mg/mL) were estimated following the standard procedures as practiced in the clinical laboratory of departments of Microbiology and Physiology of JIPMER, Pondicherry. Approximately, 0.5 mL of blood was immediately used for estimating the TLC and LC. TLC was done manually in the department of Physiology by Hemocytometry. Improved Neubauer's counting chamber and white blood cell diluting fluid

(Turk's fluid) were used for estimating the TLC. For determining the percentage distribution of lymphocytes, blood smears were made and stained with Leishman stain and then the stained blood smears were examined under oil immersion objective of the microscope and the lymphocytes were counted. Remaining blood was allowed to clot and then centrifuged to separate the serum. The serum samples were stored at −20°C in labelled containers for subsequent analyses of the following parameters:

- CD4 concentration [Rat cluster of differentiation 4, CD4 enzyme-linked immunosorbent assay (ELISA) kit, Genxbio, Cusabio][10]
- CD8 concentration (Rat cluster of differentiation 8, CD8 ELISA kit, Genxbio, Cusabio)
- Serum albumin and globulin (Biuret method, Reagent kit adapted to Agappe diagnostic, India)
- Serum Ig M (Rat Ig M ELISA kit, Genxbio, Cusabio).[11]

Using serum albumin and globulin values, albumin-globulin ratio (A-G ratio) was calculated.

Sacrifice of animals

After recording 4 weeks of postinterventional readings, all the animals were immunized on the 28th day with 1 mL of sheep red blood cells.[11] Following which all the animals were sacrificed on the 8th day of immunization as per the standard procedure described by Pal *et al*.[8]

Statistical analysis of data

For data analysis, all values were expressed as mean ± standard deviation. Differences between means were compared by Student's *t* test. The differences among means were evaluated by one-way analysis of variance using Graph pad In Stat (Version 3, USA) software. *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

Site of entry of electrode into the rat brain was noted on the brain surface [Figure 3]. Histopathological examination of rat brain section confirmed the location and extent of lesion in VMH [Figures 4-6].

Basal parameters

Basal FI and BW of male rats ($n = 12$) were 11.83 ± 0.41 g/day and 248.81 ± 0.79 g and in female rats ($n = 12$), FI and BW were 8.79 ± 0.16 g/day and 173.54 ± 0.63 g. There was a significant gender difference in FI with males eating more than females ($P < 0.001$). While comparing the BW, males weighed more than females ($P < 0.001$).

Table 1: Comparison of basal food intake, body weight, and immunological parameters of control (rats selected for sham lesion) and experimental (rats selected for ventromedial hypothalamus lesion) rats before lesion

Parameters	Control rats (*n*=12)	Experimental rats (*n*=12)	*P* value
FI (g/day)	10.23±1.87	10.40±2.43	0.8495
BW (g)	211.23±53.94	211.12±52.52	0.9960
TLC (cells/cu mm)	6482±681.65	6027.5±519.72	0.0798
LC (%)	82.03±0.52	81.43±1.74	0.2647
CD4 (pg/mL)	56.06±6.11	60.57±10.20	0.2024
CD8 (ng/mL)	1.56±0.17	1.83±0.42	0.0510
Albumin (g/dL)	3.35±0.95	3.40±1.06	0.9043
Globulin (g/dL)	4.02±0.31	3.75±0.47	0.1109
A-G ratio	0.85±0.07	0.91±0.12	0.1488

Data expressed are mean ± standard deviation. $P<0.05$ was considered significant. Analysis of data was done by Student's unpaired '*t*' test. Control means the lesion making needle electrode was introduced in to the brain but current was not passed. Experimental means the lesion making needle electrode was introduced in to the ventromedial hypothalamus and current was passed. A-G ratio: Albumin-Globulin ratio, BW: body weight, CD_4: Cluster of differentiation 4, CD8: Cluster of differentiation 8, FI: Food intake, LC: Lymphocyte count, TLC: Total leukocyte count

Control ($n = 12$) and experimental rats ($n = 12$) of VMH group had similar FI and BW before the start of experiment [Table 1]. Similarly immunological parameters like TLC, LC, CD4, CD8, albumin, globulin, and A-G ratio did not differ significantly before the start of experiment both in control and experimental rats of VMH group [Table 1].

Effect of sham lesion in control group

After undergoing sham lesions, the control male and female rats did not show any significant difference in the FI and BW from the presham values. Similarly, all the other immunological parameters like TLC, LC, CD4 concentration, CD8 concentration, albumin, globulin, and A-G ratio also did not show any significant change from the presham levels.

Effect of VMH lesion in experimental group

After the lesion, FI increased significantly in both the sexes ($P < 0.001$) [Table 2], but the percentage increase in FI was more in females than males [Figure 1]. Both males and females showed an increase in BW, but was significantly more in males ($P < 0.001$) [Table 2 and Figure 1]. After VMH lesion, both male and female rats showed decrease in CD4 concentration but the decrease in CD4 concentration was significant only in male rats ($P < 0.001$). Also, there was a significant decrease in albumin levels ($P < 0.01$) in both male and female rats, following VMH lesion [Table 2 and Figure 1]. Rest of the parameters namely TLC, LC, CD8, globulin, A-G ratio, LW-BW ratio, SW-BW ratio, and Ig M did not show any significant change, following VMH lesion [Table 2].

Table 2: Food intake, body weight, and immunological parameters in experimental male (*n*=6) and female (*n*=6) rats before (prelesion) and after (postlesion) ventromedial hypothalamus lesion

Parameters	Prelesion		Postlesion	
	Male	Female	Male	Female
FI (g/day)	12.12±0.47	8.68±0.21***	15.33±0.94***	13.17±0.80###,fff
BW (g)	248.26±24.27	173.99±11.05***	294.46±14.41***	191.39±14.11***,fff
TLC (cells/cu mm)	6395±566.35	5660±1201.8	6291.67±492.36	6630±892.75
LC %	82.67±4.55	80.2±4.28	84±5.06	80±1.73
CD4 (pg/mL)	67.79±10.54	53.36±7.26*	38.09±9.22***	40.18±5.11***
CD8 (ng/mL)	1.86±0.74	1.80±0.85	1.98±1.00	1.00±0.55
Albumin (g/mL)	3.47±0.10	3.42±0.18	2.95±0.35**	2.96±0.18**,##
Globulin (g/dL)	3.42±0.6	4.08±0.62	2.77±0.41	3.4±0.21
A-G ratio	1.04±0.16	0.86±0.06	1.09±0.15	0.94±0.09
LW-BW ratio	NA	NA	0.031±0.0024	0.033±0.0037
SW-BW ratio	NA	NA	0.0032±0.0007	0.0034±0.0005
Ig M (mg/mL)	NA	NA	0.25±0.14	0.16±0.04

Data expressed are mean ± standard deviation. The (*) represents comparison with prelesion male, (#) represents comparison with prelesion female, (f) represents comparison with postlesion male. The analysis of data was done by one-way analysis of variance and post hoc by Tukey-Kramer test except for liver weight-body weight ratio, spleen weight-body weight ratio, and immunoglobulin M. Analysis of liver weight-body weight ratio, spleen weight-body weight ratio, and immunoglobulin M was done by Student's unpaired '*t*' test. **P*<0.05; ***P*<0.01; ****P*<0.001. A-G ratio: Albumin-globulin ratio, BW: Body weight, CD4: Cluster of differentiation 4, CD8: Cluster of differentiation 8, FI: Food intake, Ig M: Immunoglobulin M, LC: Lymphocyte count, LW-BW ratio: Liver weight-body weight ratio, NA: Not applicable, SW-BW ratio: Spleen weight-body weight ratio, TLC:Total leukocyte count, Lesion means the lesion making needle electrode was introduced in to the ventromedial hypothalamus and current was passed

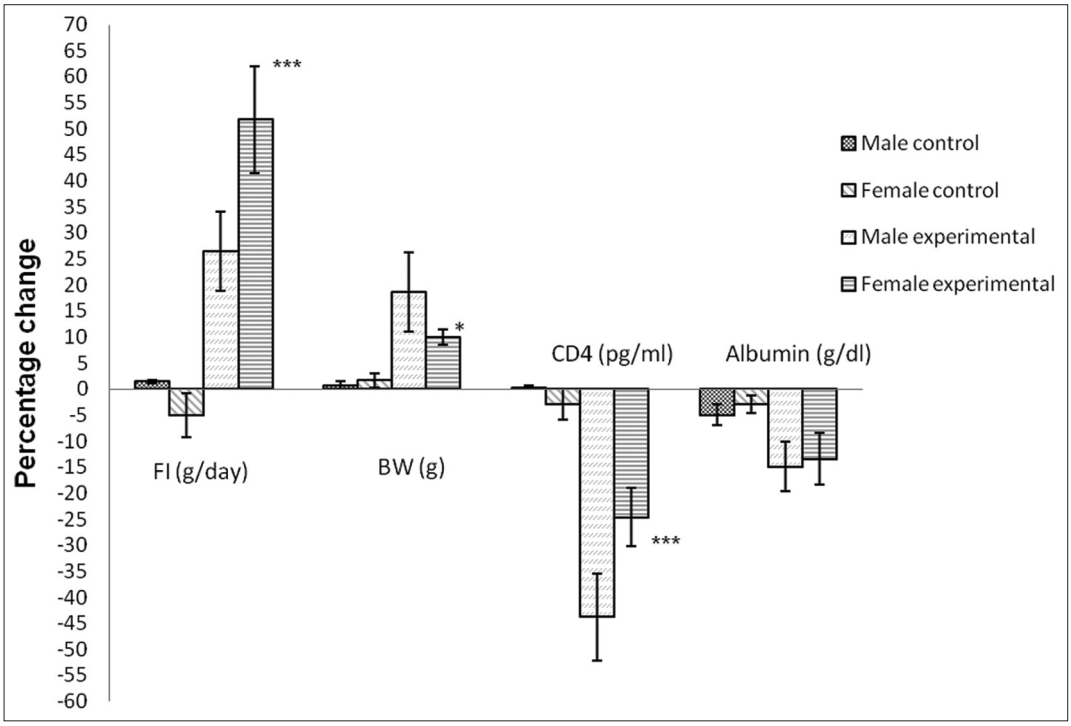

Figure 1: Male–female difference of mean percentage change in experimental and control rats of ventromedial hypothalamus (VMH) group, following lesion of VMH compared with their prelesion values. Control means the lesion making needle electrode was introduced in to the brain but current was not passed. Experimental means the lesion making needle electrode was introduced in to the ventromedial hypothalamus and current was passed. BW: Body weight, CD4: Cluster of differentiation 4, FI: Food intake. (*) represents comparison with males.**P* < 0.05; ***P* < 0.01;****P* < 0.001

DISCUSSION

In the present study, following VMH lesion there was a significant increase in FI in both experimental male and female rats (*P* < 0.001) compared with the control rats [Table 2]. This confirms the inhibitory nature of VMH on FI, for which VMH has been designated as satiety center.[4,7] However, the percentage increase in FI was more in females than the males indicating that VMH has a greater effect on FI in females compared

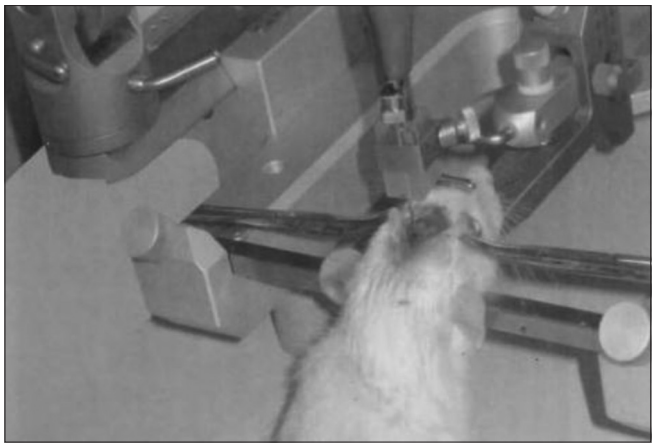

Figure 2: The picture depicting head of the anesthetized rat fixed in the stereotaxy apparatus by fixing with the help of biaural head fixation system of the apparatus

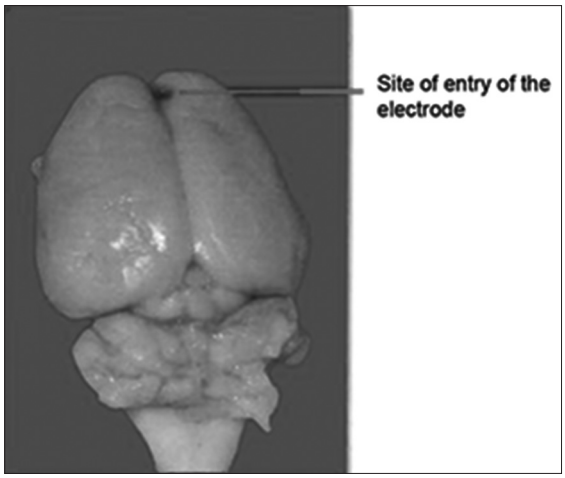

Figure 3: The picture of the brain of the rat showing the site of lesion making electrode entering the brain to get access into ventromedial hypothalamus

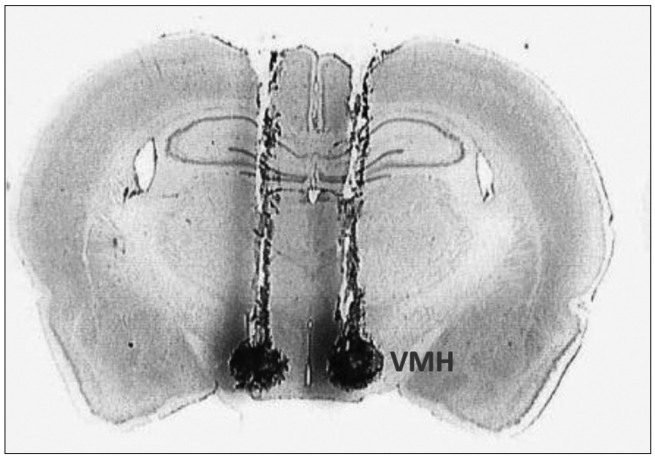

Figure 4: Brain histology [section through ventromedial hypothalamus (VMH)] showing the placement of electrodes and lesion of VMH on both the sides. The electrodes were uninsulated to show the tract of its passage from the top of the brain to the VMH nucleus. Electric current was passed to demonstrate the tract and the nuclei damaged by the lesion on both sides

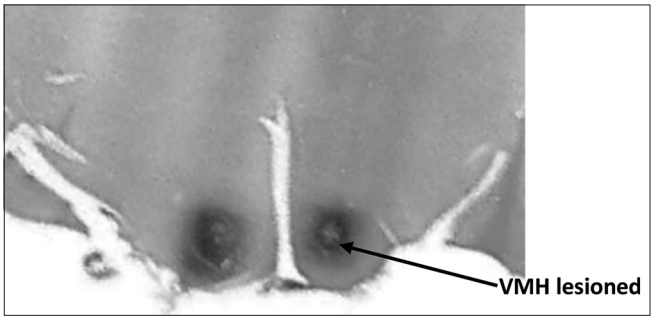

Figure 5: Rat brain section through ventromedial hypothalamus (VMH) showing the bilateral electrolytic lesion of VMH

with male rats [Figure 1]. Therefore, the presence of a significant male-female difference in FI of control group (sham-lesion rats) disappeared after VMH lesion.

Following VMH lesion, there was increase in the BW in both experimental male and female rats compared with the control rats and the increase in BW was significant in male rats ($P < 0.001$) [Table 2] in spite of FI being more in females. This confirms VMH as major nuclei regulating FI and BW. The male-female difference in BW following lesion was highly significant ($P < 0.001$), which could be due to the prevailing gender difference in BW before lesion ($P < 0.001$). Lesion of VMH leads to obesity which is referred to as ''hypothalamic obesity.''[12] Therefore, in our study, following VMH lesion the BW gain was about 18.6% in males and 10% in females, respectively.

VMH lesion results in increased parasympathetic activity and decreased sympathetic activity.[6,13] Sympathetic activation causes BW loss and parasympathetic activation causes BW gain. Hence, energy homeostasis is mainly regulated by sympathovagal balance.[14] Presently, it is strongly believed that alteration in energy homeostasis in VMH-lesion is mainly due to neural influence driven by decreased sympathetic and increased parasympathetic activity, not due to hyperphagia.[15] Though the reports on gender difference in obesity induced by VMH lesion are not concretely available, some findings indicate lesser degree of obesity in female. Findings of present study clearly indicate less increase in BW gain in female rats, following VMH lesion [Table 2]. Our findings corroborate with the report of Dev *et al*.,[7] that satiation effect induced by VMH in females is more than that of the males and hence VMH is closely linked to energy balance of the body. This gender difference could be principally due to the difference in the degree of sympathovagal output following VMH lesion, in which inhibition in sympathetic output may be less in female rats compared with that of male rats leading to a relatively higher sympathetic discharge in females.

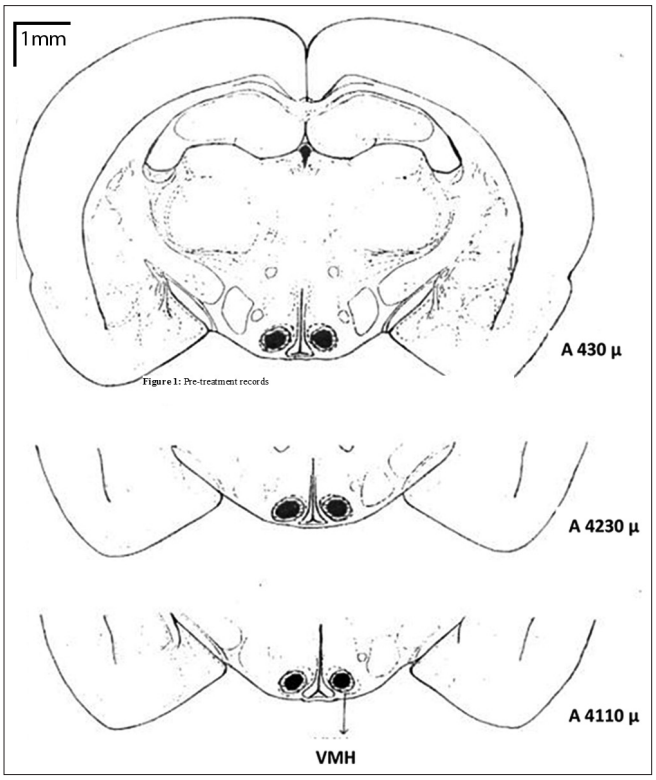

Figure 6: Reconstruction diagram of rat brain sections at the level of ventromedial hypothalamus (VMH) showing minimum (dark area) and maximum (dark and the outer area in the circle) of extent of lesion

Important immunological parameters recorded in the present study are TLC, LC, CD4 concentration, CD8 concentration, albumin, globulin, A-G ratio, LW-BW ratio, SW-BW ratio, and Ig M. In the present study, before VMH lesion, there was significantly less CD4 level in females compared with males, though the decrease in CD8 concentration in females was not statistically significant. This indicates that cellular immunity is normally less in females, as CD4 mainly represents cellular immunity.[16,17] This corroborates with the report of Afshan *et al*.,[18] which indicates lesser cellular immunity in females. However, following VMH lesion there was reduction in CD4 concentration, which was more prominent in males compared to females. This shows that the cellular immunological response in males is more suppressed following VMH lesion, indicating that VMH as an important hypothalamic structure for activation of immunological responses in rats. Our findings corroborate with the report of Wrona[19] that medial hypothalamus could be among the important brain areas for immunomodulation. This could be due to a greater influence of VMH on hypothalamo-pituitary-immune response in males compared with the females.[19,20]

The serum level of albumin was significantly decreased in both males and females following VMH lesion and there was no gender difference in the change in albumin concentration. This indicates that VMH has stimulatory effect on nonspecific immunity which has no gender specificity. There is no much difference in globulin, A-G ratio, and Ig M between the groups, indicating that the effect of lesion on humoral immune responses was not significant. These findings clearly depict the dissociation in hypothalamic neural control mechanisms on FI and adiposity in rats and their difference on the influence of gender in hypothalamic modulation of immunity.

CONCLUSION

The lesion of VMH results in accentuation of FI and BW gain, and suppression in cell-mediated immunity. The effect of increased FI was more in females and increased BW gain was more in males, indicating that inspite of less influence on control of FI in males, VMH has a stronger regulation of adiposity in males. Also, VMH control of immunological responses is more pronounced in males.

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