

Original Article

# Comparison of the effect of lesions of ventromedial hypothalamus and posterodorsal amygdala on body weight and immunological parameters in albino Wistar rats

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## Abstract

**Background and Aim:** Although hypothalamic and extrahypothalamic areas are known to influence food intake (FI), body weight (BW) and immunity, the exact nature and magnitude of alteration following lesion of these areas have not adequately studied. Therefore, the present study was aimed at comparing the effect of lesions of ventromedial hypothalamus (VMH) and posterodorsal amygdala (PDA) on FI, BW gain and immunological parameters in albino Wistar rats.

**Methods:** A total of 48 albino Wistar rats were taken for the study and were divided equally into VMH group and PDA group with 12 control and 12 experimental rats in each. Bilateral electrolytic lesion of the respective nuclei was performed by stereotaxy. The pre- and post-lesion parameters of both groups were compared.

**Results:** The percentage increase in FI and BW gain was significantly less ( $P < 0.001$ ) in the experimental rats of PDA group. There was a significant difference in the percentage change in cluster of differentiation 4 (CD4) and CD8 concentration ( $P < 0.001$ ) in experimental rats of PDA group compared with the experimental rats of VMH group. The percentage decrease in albumin and globulin ( $P < 0.001$ ) levels and the percentage increase in albumin-globulin ratio ( $P < 0.001$ ) was significantly less in experimental rats of PDA group.

**Conclusion:** The above-mentioned findings suggest that the role of VMH on feeding is more pronounced than PDA, indicating that VMH has a stronger regulation of adiposity than PDA. Though VMH and PDA are involved in the regulation of immune functions, VMH has a stronger control over immune functions than PDA. Hence, VMH has greater control over adiposity, feeding behavior, and immune functions than PDA.

**Key words:** Body weight, food intake, hypothalamus, immunity, posterodorsal amygdala, ventromedial

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## INTRODUCTION

Food intake (FI) is controlled by a complex system of both central and peripheral signals that interact to alter the individual response to feeding. The peripheral regulation includes the satiety signals, and the central control is by the various neurotransmitters acting on the different brain areas.<sup>[1]</sup> The hypothalamus is the major regulator of FI and body weight (BW) gain.<sup>[2]</sup> Previous studies have reported

that destruction of ventromedial hypothalamus (VMH) produces severe hyperphagia, obesity and alteration in immunity, showing that VMH is the key center for the regulation of adiposity and immunological parameters.<sup>[3-5]</sup>

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The extra-hypothalamic areas such as amygdala, nucleus septal medialis, nucleus accumbens, and caudate nucleus also influence the feeding behavior.<sup>[6]</sup> There are reports showing that posterodorsal amygdala (PDA) is involved in the regulation of adiposity and immunity.<sup>[7-10]</sup> Hence, both hypothalamic areas and extra-hypothalamic areas are known to influence FI, BW, and immunity.<sup>[3-5,7,11,12]</sup> However, till date, only very few studies have been done so far to compare the role of hypothalamus and extra-hypothalamic areas on the regulation of adiposity and immunity. Moreover, the exact nature of change and the magnitude of alteration in adiposity and immunological parameters following lesions of VMH and PDA have not been fully studied so far. Therefore, in the present study, we have planned to compare the effect of lesions of VMH and PDA on the regulation of adiposity and immunological parameters in rat models.

## MATERIALS AND METHODS

### Animals

After the approval of the Research Council and Animal Ethics Committee of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), a total of 48 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 of Physiology department, 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 food intake and body weight 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 (bilateral lesion made in VMH; 12 control and 12 experimental rats)
- PDA group (bilateral lesion made in PDA; 12 control and 12 experimental rats).

### Procedures

#### Anesthesia

Different anesthetic agents were used because the depth of anesthesia required for different procedures differed. For blood collection, light anesthesia is required; hence,

ether was used as the anesthetic agent for this purpose. Injection ketamine (0.25 ml/250 g BW) was injected intraperitoneally for making lesion, and for sacrificing the animal double the dose of ketamine was injected intraperitoneally as described by Dev *et al.*<sup>[4]</sup>

#### Blood collection

For obtaining the basal immunological values, 1.5–2 ml of blood was collected by Jugular venous puncture after obtaining 7 days of basal readings of FI and BW. 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 the brain.

#### Electrolytic nuclear lesion

The stereotaxic procedure was performed as described by Pal *et al.*<sup>[13]</sup> for making the brain lesions. Bilateral electrolytic lesions of VMH and PDA were made by introducing the electrodes into the respective nuclei on both sides according to the following coordinates (PDA: anterior: 0.33 cm, lateral:  $\pm 0.46$  cm, vertical: 0.72 cm; VMH: Anterior: 0.45 cm, lateral:  $\pm 0.05$  cm, vertical: 0.82 cm) obtained from the stereotaxic atlas of rat brain by König and Klippel,<sup>[14]</sup> 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 14 days during which FI and BW were not measured.

##### Immunological parameters

Immunological parameters namely cluster of differentiation 4 (CD4) concentration (pg/ml), CD8 concentration (ng/ml), serum albumin (g/dl), serum globulin (g/dl), albumin-globulin (A-G) ratio, liver weight (LW)-BW ratio, spleen weight-BW (SW-BW) ratio, and serum immunoglobulin M (IgM) (mg/ml) were estimated following the standard procedures as practiced in the clinical laboratory of Departments of Microbiology and Physiology of JIPMER, Puducherry. Approximately, 5 ml of blood was allowed to clot and then centrifuged to separate the serum. The serum samples were stored at

–20°C in labeled containers for subsequent analyses of the following parameters:

1. CD4 concentration (Rat cluster of differentiation 4, CD4 ELISA kit, Genxbio, Cusabio)<sup>[15]</sup>
2. CD8 concentration (Rat cluster of differentiation 8, CD8 ELISA kit, Genxbio, Cusabio)
3. Serum albumin and globulin (Biuret method, Reagent kit adapted to Agappe diagnostic, India)
4. Serum IgM (Rat IgM ELISA kit, Genxbio, Cusabio)<sup>[12]</sup>
5. Using serum albumin and globulin values, A-G ratio was calculated.

### Sacrifice of animals

After recording 4 weeks of postinterventional readings, all the animals were immunized on the 28<sup>th</sup> day with 1 ml of sheep red blood cells.<sup>[12]</sup> Following which all the animals were sacrificed on the 8<sup>th</sup> day of immunization as per the standard procedure described by Pal *et al.*<sup>[13]</sup> The liver and spleen were removed and weighed. LW-BW ratio and SW-BW ratio were measured.

### Statistical analysis of data

For data analysis, all values were expressed as mean  $\pm$  standard deviation. Differences between means were compared by Student's *t*-test using GraphPad InStat (Version 3, USA) software. The difference was considered statistically significant if probability of chance was  $<0.05$  ( $P < 0.05$ ).

## RESULTS

Table 1 shows the comparison of FI, BW and immunological parameters between control rats of VMH and PDA group before and after sham lesion. Before the start of the experiment, the control rats of VMH and PDA group had similar FI and BW. Immunological parameters like CD4

concentration, CD8 concentration, and albumin did not show any significant change before the start of experiment between the control rats of VMH and PDA group; however, there was a significant difference in globulin level ( $P < 0.01$ ) and A-G ratio ( $P < 0.001$ ). Following sham lesion of respective nuclei, the control rats of both VMH and PDA group did not show any significant change in FI, BW, and immunological parameters such as CD8 concentration and albumin levels. The significant difference which was observed in the globulin level ( $P < 0.01$ ) and A-G ratio ( $P < 0.001$ ) between the control rats of VMH and PDA group before the start of experiment remained unchanged even after their respective sham lesions. There was no significant difference in LW-BW ratio, SW-BW ratio, and IgM between the control rats of VMH and PDA group.

Table 2 shows the effect of lesions of VMH and PDA on FI, BW, and immunological parameters between the control and experimental rats of VMH and PDA group. The experimental rats of VMH and PDA group had similar FI and BW before the lesion and similarly, immunological parameters such as CD4 concentration, CD8 concentration, albumin, globulin, and A-G ratio did not differ significantly before the start of experiment. Following lesion [Figure 1 and 2], though there was a difference in the FI, BW, and immunological parameters such as CD8 concentration, globulin level, A-G ratio, and IgM between the experimental rats of VMH and PDA, the difference was not significant. There was a significant difference in the CD4 concentration ( $P < 0.001$ ), albumin level ( $P < 0.001$ ), LW-BW ratio ( $P < 0.05$ ), and SW-BW ratio ( $P < 0.01$ ) in the experimental rats of VMH group compared to PDA group following lesion.

Table 3 shows the mean percentage change in FI, BW, and immunological parameters of control and experimental rats of both VMH and PDA group. There was no significant

**Table 1:** Comparison of food intake, body weight, and immunological parameters between control rats (rats selected for sham lesion) of both ventromedial hypothalamus and posterodorsal amygdala group before and after sham lesion of their respective nuclei

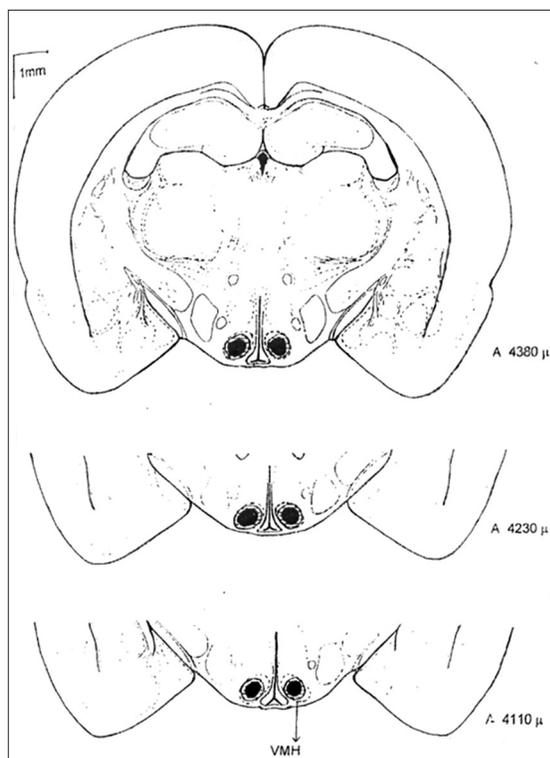
Parameters	Control rats before sham lesion (n=12)			Control rats after sham lesion (n=12)		
	VMH	PDA	P	VMH	PDA	P
FI (g/day)	10.23 $\pm$ 1.87	9.54 $\pm$ 3.23	0.5285	10.08 $\pm$ 2.31	9.65 $\pm$ 2.81	0.6861
BW (g)	211.23 $\pm$ 53.94	209.83 $\pm$ 72.24	0.9576	213.65 $\pm$ 53.22	209.94 $\pm$ 72.38	0.8876
CD4 (pg/ml)	56.06 $\pm$ 6.11	58.37 $\pm$ 1.66	0.2195	55.39 $\pm$ 7.36	59.80 $\pm$ 0.18	0.0499
CD8 (ng/ml)	1.56 $\pm$ 0.17	1.98 $\pm$ 1.38	0.3067	1.45 $\pm$ 0.60	1.61 $\pm$ 0.66	0.5407
Albumin (g/dl)	3.35 $\pm$ 0.95	3.29 $\pm$ 0.18	0.8318	3.22 $\pm$ 0.09	3.24 $\pm$ 0.13	0.6655
Globulin (g/dl)	4.02 $\pm$ 0.31	3.40 $\pm$ 0.50**	0.0014	3.57 $\pm$ 0.42	3.08 $\pm$ 0.28**	0.0028
A-G ratio	0.85 $\pm$ 0.07	1.03 $\pm$ 0.12***	0.0002	0.94 $\pm$ 0.05	1.07 $\pm$ 0.06***	<0.0001
LW-BW ratio	NA	NA	NA	0.035 $\pm$ 0.007	0.033 $\pm$ 0.004	0.3994
SW-BW ratio	NA	NA	NA	0.0035 $\pm$ 0.0007	0.0034 $\pm$ 0.0006	0.7107
IgM (mg/ml)	NA	NA	NA	0.26 $\pm$ 0.02	0.27 $\pm$ 0.03	0.3471

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Data expressed are mean $\pm$ SD. Analysis of data was done by Student's unpaired *t*-test, The (\*) represents comparison between VMH and PDA control rats, control means the lesion making needle electrode was introduced in to the brain but current was not passed. A-G ratio: Albumin-globulin ratio, BW: Body weight, CD4: Cluster of differentiation 4, CD8: Cluster of differentiation 8, FI: Food intake, IgM: Immunoglobulin M, LW-BW ratio: Liver weight-body weight ratio, SW-BW ratio: Spleen weight-body weight ratio, SD: Standard deviation, NA: Not available, VMH: Ventromedial hypothalamus, PDA: Posterodorsal amygdala

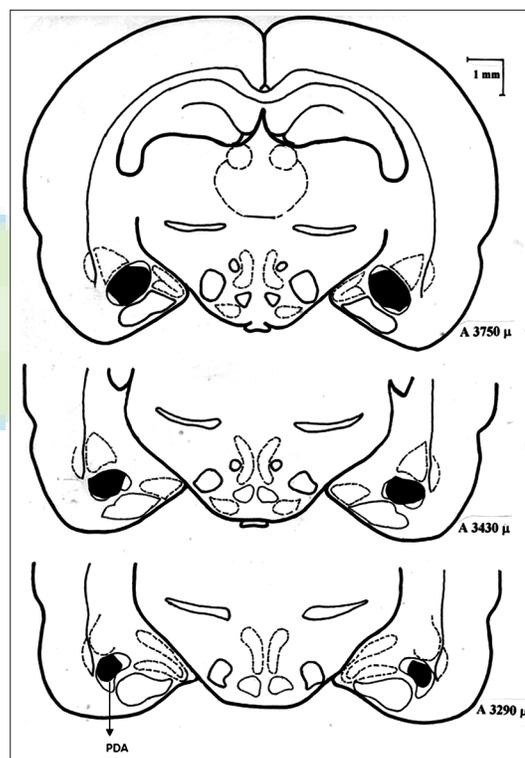
**Table 2:** Comparison of food intake, body weight, and immunological parameters of experimental rats (rats selected for lesion of respective nuclei) of both ventromedial hypothalamus and posterodorsal amygdala group before and after lesion

Parameters	Experimental rats before lesion (n=12)			Experimental rats after lesion (n=12)		
	VMH	PDA	P	VMH	PDA	P
FI (g/day)	10.40±2.43	9.64±2.60	0.4673	14.25±1.53	13.13±3.02	0.2641
BW (g)	211.12±52.52	201.88±56.01	0.6808	242.93±72.88	225.65±72.86	0.5672
CD4 (pg/ml)	60.57±10.20	57.72±0.90	0.3454	39.14±1.48	63.39±7.00 <sup>fff</sup>	<0.0001
CD8 (ng/ml)	1.83±0.42	1.94±0.67	0.6347	1.49±0.89	2.54±1.81	0.0850
Albumin (g/dl)	3.40±1.06	3.34±0.13	0.8475	2.96±0.007	3.17±0.07 <sup>fff</sup>	<0.0001
Globulin (g/dl)	3.75±0.47	3.57±0.31	0.2801	3.09±0.45	3.25±0.17	0.2616
A-G ratio	0.91±0.12	0.94±0.04	0.4201	1.02±0.11	0.99±0.01	0.3570
LW-BW ratio	NA	NA	NA	0.03±0.001	0.035±0.007 <sup>f</sup>	0.0227
SW-BW ratio	NA	NA	NA	0.0035±0.0002	0.0038±0.0003 <sup>f</sup>	0.0086
IgM (mg/ml)	NA	NA	NA	0.21±0.06	0.22±0.01	0.5748

<sup>f</sup>P<0.05, <sup>ff</sup>P<0.01, <sup>fff</sup>P<0.001. Data expressed are mean±SD. Analysis of data was done by Student's unpaired t-test. The (f) represents comparison between VMH and PDA experimental rats, Experimental means the lesion making needle electrode was introduced in to the respective nuclei and current was passed. A-G ratio: Albumin-globulin ratio, BW: Body weight, CD4: Cluster of differentiation 4, CD8: Cluster of differentiation 8, FI: Food intake, IgM: Immunoglobulin M, LW-BW ratio: Liver weight-body weight ratio, SW-BW ratio: Spleen weight-body weight ratio, SD: Standard deviation, NA: Not available, VMH: Ventromedial hypothalamus, PDA: Posterodorsal amygdala



**Figure 1:** Reconstruction diagram of rat brain sections at the level of ventromedial hypothalamus showing the minimum (dark area) and maximum (dark and outer area in the circle) of the extent of lesion



**Figure 2:** Reconstruction diagram of rat brain sections at the level of posterodorsal amygdala showing the minimum (dark area) and maximum (dark and outer area in the circle) of the extent of lesion

difference in the mean percentage change between the VMH and PDA control rats following sham lesion in all the parameters. The percentage increase in FI ( $P < 0.001$ ) and BW gain ( $P < 0.001$ ) was significantly less in experimental rats of PDA group compared to the experimental rats of VMH group. There was a significant difference in the percentage change in CD4 and CD8 concentration ( $P < 0.001$ ) in experimental rats of PDA group compared

with the experimental rats of VMH group; the experimental rats of VMH group showed a decrease in CD4 and CD8 concentration, whereas the experimental rats of PDA group showed an increase in CD4 and CD8 concentration. The percentage decrease in albumin and globulin ( $P < 0.001$ ) levels and the percentage increase in A-G ratio ( $P < 0.001$ ) was significantly less in experimental rats of PDA group compared to the experimental rats of VMH group.

## DISCUSSION

Our present work was aimed at examining the possible involvement of VMH and PDA in the control of FI, BW gain and immunity. Previous studies have confirmed the inhibitory nature of VMH on FI and BW gain.<sup>[2-4]</sup> Similarly, PDA lesion also resulted in an increase in FI and BW gain.<sup>[8-10]</sup> However, the gain in BW by VMH rats was more pronounced than the PDA rats [Table 3]. This confirms the inhibitory control of VMH on FI and BW gain is more pronounced when compared with that of PDA.

While comparing the immunological parameters, VMH lesion decreased both cell-mediated (CD4 concentration: -35.38% and CD8 concentration: -18.58%) and humoral immunity (albumin: -12.94% and globulin: -17.6%) [Table 3]. The decrease in cell-mediated immunity was quite huge in CD4 concentration; almost one-third from the prelesion value and the decrease in CD8 concentration was about one-fifth from their prelesion value. This shows that normally VMH has a stimulatory effect on both cell-mediated and humoral immunity. Lesion of PDA resulted in increase in cell-mediated immunity (CD4 concentration: 9.82% and CD8 concentration: 30.92%) and decrease in humoral immunity (albumin: -5.09% and globulin: -8.96%) [Table 3]. The increase in CD8 concentration was quite impressive (almost 30% from their prelesion value) when compared with that of CD4 concentration, whereas the decrease in humoral immunity was negligible (5% and 8% decrease only). This shows that PDA normally has an immunosuppressive effect primarily on cellular immunity. Hence, we observed that normally VMH stimulates both cellular and humoral immunity; whereas, PDA suppresses the cellular immunity.

The degree of alteration in FI, BW, and immunological parameters in general was more prominent in VMH

lesioned rats compared to that of PDA lesioned rats. One should note that the obesity induced by PDA has not resulted in immunosuppression as was observed following VMH lesion. It could be that the obesity-induced immunosuppression was overshadowed by the immune activation by PDA lesion. It may also be due to the lesser degree of adiposity produced by PDA lesion in comparison to adiposity induced by VMH lesion.

From this study, it appears that VMH is more effectively involved in the control of FI and BW gain than PDA. Hence, VMH and PDA are a part of the same ipsilateral pathway regulating feeding behavior and BW.<sup>[16,17]</sup> While comparing the immunological parameters, here also, VMH has a much stronger role on immunity than PDA, the impact of VMH on immunity is stimulatory, and the impact of PDA on immunity is inhibitory.

### Limitations of the study

The neurotransmitters in VMH and PDA lesioned rats have not been estimated and their influence on body weight and immunity parameters have not been assessed.

## CONCLUSION

VMH and PDA are part of an inhibitory pathway in the regulation of feeding behavior. The role of VMH on feeding is more pronounced than PDA, indicating that VMH has a stronger regulation of adiposity than PDA. Though VMH and PDA are involved in the regulation of immune functions, VMH has a stronger control over immune functions than PDA. However, the nature of interaction between VMH and PDA should be further explored.

**Table 3:** Comparison of mean percentage change (from their prelesion values) in food intake, body weight, and immunological parameters of postlesion control and experimental rats of both ventromedial hypothalamus and posterodorsal amygdala groups

Parameters	Control rats (n=12)			Experimental rats (n=12)		
	VMH	PDA	P	VMH	PDA	P
FI (g/day)	-1.47±2.98	1.15±3.64	0.0667	37.02±0.46	36.20±0.22 <sup>fff</sup>	<0.0001
BW (g)	1.15±2.24	0.05±1.56	0.1767	15.07±1.22	11.77±1.68 <sup>fff</sup>	<0.0001
CD4 (pg/ml)	-1.20±4.42	2.44±4.76	0.0652	-35.38±2.22	9.82±4.24 <sup>fff</sup>	<0.0001
CD8 (ng/ml)	-7.05±8.56	-18.68±20.4	0.0825	-18.58±2.26	30.92±4.84 <sup>fff</sup>	<0.0001
Albumin (g/dl)	-3.88±2.99	-1.52±2.86	0.0608	-12.94±2.42	-5.09±1.68 <sup>fff</sup>	<0.0001
Globulin (g/dl)	-11.19±8.62	-9.41±7.24	0.5894	-17.6±0.32	-8.96±0.46 <sup>fff</sup>	0.0016
A-G ratio	10.59±9.62	3.88±5.94	0.0518	12.08±0.34	5.32±0.21 <sup>fff</sup>	<0.0001

<sup>f</sup>P<0.05, <sup>ff</sup>P<0.01, <sup>fff</sup>P<0.001. Data expressed are mean±SD. Analysis of data was done by Student's unpaired t-test, The (f) represents comparison between VMH and PDA experimental rats, 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 respective nuclei and current was passed. A-G ratio: Albumin-globulin ratio, BW: Body weight, CD4: Cluster of differentiation 4, CD8: Cluster of differentiation 8, FI: Food intake, SD: Standard deviation, NA: Not available, VMH: Ventromedial hypothalamus, PDA: Posterodorsal amygdala

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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