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NEURO IMMUNO MODULATION BY VENTRAL HIPPOCAMPUS

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Abstract: Bilateral electrical lesion at ventral hippocampal formation (VHF) did not affect some aspects of non-specific immunity like total W.B.C. count, percentage of cells in differential count, their absolute count (lymphocyte and neutrophils) and neutrophil functions. The changes observed are due to non-specific craniocerebral trauma as the sham operated animals also showed a similar pattern of response. However the lesion at VHF increases the spleen cell count significantly whereas immunization in these animals decreases the spleen cell count. The thymus weight/body weight ratio also decreases in these animals. Our study confirms the neuroimmuno modulation and the influence of VHF on certain non-specific immune parameters.

Key words : neuro immuno modulation hippocampal lesion non-specific immunity

INTRODUCTION

Recently considerable data and evidence support that the immune system is not as autonomous as once believed. Destructive lesions in the hypothalamus result in marked alterations in the normal cellular architecture of lymphoid tissue (1, 2) diminished delayed type hypersensitivity reaction (3, 4) and impaired humoral antigenic responsiveness (5, 6). Moreover, increase in electrical aciv-ity was recorded in the ventro medical nucleus of hypothalamus during the peak of the immune response (7) and in the preoptic and the anterior hypothalamus on the 5th day after SRBC injection. The increased activity corrlelates with initial appearance of antibodies (8). This indicates the flow of information from the activated immune system to the hypothalamus and implicates the brain in immune process.

However, the influence of hippocampus on the immune mechanisms is not clearly established. Hence, the present study was undertaken to elucidate the influence of VHF on some non-specific immune parameters.

Male Wistar strain albino rats weighing 180-190 g were used in these experiments. They were fed ad libitum with standard laboratory feed and water and were housed under standard laboratory conditions.

The animals were devided into 6 groups as follows:

Group I : Normal control rats (n = 15). These animals provided normal basal value for the immune parameters studied.

Group II : Immunized control rats (imm.) (n = 15). These rats were immunized with sheep red blood cells (SRBC). This group gave the normal response of animals for this particular dose of antigen.

Group III : VHF lesioned rats (n = 7). This group was used to detect the change in basal immune status due to the lesion.

Group IV : VHF lesioned animals, immunized with SRBC (n = 7). This group was used to detect the effect of lesion on the immune response.

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Group V : VHF sham operated animals (n = 7). This group was used to elucidate the effect of non-specific stress and surgical injury influencing the immune mechanism.

Group VI : VHF sham immunized animals (n = 7) were used to elucidate the effect of non-specific stress and surgical injury influencing the immune mechanism during an immune response.

Bilateral lesions and sham operations were performed according to Konig and Klippel (9) atlas. The co-ordinates were 2.58 mm anterior, 4 mm lateral and 7.6 mm deep from dura. Electrolytic lesion was produced by coated stainless steel electrode with 0.22 mm dia exposed only at the tip. Direct current of 2 mA for 45 sec. was used. The sham lesioned animals were subjected to the same procedure except the passage of current. There was no mortality and all the animals appeared healthy.

Immunization : To immunize, 5×10^9 SRBC/ml was injected intraperitoneally. The day of immunization was the 'O' day. In sham and lesion animals, immunization was carried out on the 10th day after surgery. The parameters were studied on the 14th and 15th day in groups III, IV, V and VI and on 4th and 5th day in control immunized animals (group I). In each animal the following parameters were studied:

1. Total W.B.C. count,
2. Differential count and absolute count (lymphocyte and neutrophil),
3. Neutrophil function test
   (a) Candida phagocytosis (10)
   (b) Nitroblue tetrazolium reduction (NBT) (11)
4. Organ weight/body weight ratio of spleen thymus and popliteal lymph node.
5. Cell count in spleen and thymus
6. Soluble immune complex (12).

Heparinized blood was collected from the jugular vein. At the time of sacrifice the brain of each lesioned animal was removed and the site and extent of lesion was confirmed histologically using cresyl fast violet staining. The spleen, thymus and lymph nodes were removed aseptically and weighed. Cell suspension was prepared for cell count from spleen and thymus in MEM AT 045 medium.

All the results were analysed in various combination permutation using Students' 't' test.

RESULTS

The total WBC count/cu. mm. (TWBC) of various groups are given in Table I. Though the total WBC count in control immunized ($P<0.001$), lesion ($P<0.001$), and sham ($P<0.001$) animals showed a significant fall from control values, the sham group TWBC count was not significantly different from the lesion, and TWBC of sham immunized group was similar to the total count of lesion immunized animals. Therefore it can be safely concluded that these effects were not specifically due to lesion.

TABLE I : The total WBC count/cu mm is expressed as Mean ± S.D.

Groups	*Counts/cu mm*
Control rats (Group I, n = 15)	16706 ± 1669
Control immunized (Group II, n = 15)	13953 ± 1452
Lesion (Group III; n = 7)	13171 ± 795
Lesion immunized (Group IV; n = 7)	11428 ± 941
Sham (Group V; n = 7)	11428 ± 1382
Sham immunized (Group VI; n = 7)	13792 ± 646

The percentage of polymorphs and its absolute count/cu m.m. is given in Table II.

The polymorph percentage and absolute count showed a significant decrease in immunized control ($P<0.001$), VHF sham ($P<0.001$) and VHF lesion ($P<0.001$) animals compared to control group I. VHF sham immunized group showed a significant fall ($P<0.05$) in absolute neutrophil count compared to control immunized group.

The VHF lesion group showed a significant increase ($P<0.01$) in absolute neutrophil count compared to VHF sham which points to a specific lesion effect.

TABLE II : Differential count in test and control animals.

	Groups	Polymor		Lymphocyte		Eosinopil		Monocyte	Basophil
		%	Absolute count	%	Absolute count	%	Absolute count	%	%
I	Control rats (n = 15)	27.0±5.15	4494±810.8	67.9±6.38	11336±1398	3.4±2.58	609.7±481.9	1.3±0.789	0.3±0.48
II	Control immunized (n = 15)	17.0±3.26	2379±505.7	80.0±3.15	11232±295	1.2±1.9	311.25±305	1.0±0.75	0.13±0.35
III	Lesion (n = 7)	17.4±2.9	2297±424.0	76.0±55.48	10009±946	3.43±2.46	456±378	0.86±0.9	0.71±0.76
IV	Lesion immunized (n = 7)	17.4±3.5	2320±452.0	76.57.5.4	10235±5.4	3.86±2.4	512±301	1.29±0.76	0.86±0.69
V	Sham (n = 7)	12.86±1.95	1467±292.0	82.0±4.32	9375±1252	3.29±2.69	381±3.19	1.43±0.53	0.7±0.49
VI	Sham immunized n = 7)	14.0±3.0	1924±381.0	81.5±3.5	11256±810	3.29±1.5	452±202	1.0±0.58	0.14±0.38

The percentage of cells in peripheral blood smear and absolute counts of neutrophil, polymorph and eosinophil are given as mean + S.D.

The percentage of lymphocytes and its absolute count were given in Table II.

The lymphocyte percentage was increased significantly in control immunized (P<0.001) VHF sham (P<0.001) and VHF lesion (P<0.01) when compared with controls, whereas the absolute lymphocyte count showed no significant change in control immunized animals. VHF lesion (P<0.05) and VHF sham (P<0.01) showed a decrease when compared with controls. But the changes observed in sham and sham immunized group did not significantly differ from lesion and lesion immunized groups. Hence the changes are not due to lesion.

Except the increase in percentage of eosinophils in VHF sham immunized (P<0.02) when compared with control immunized group, the changes observed in eosinophils, basophils and monocytes were not much of significance due to the larger individual variation.

Neutrophil function test was assessed by two tests—phagocytic activity by candida phagocytosis and intracellular killing power by NBT reduction test. The results of phagocytic activity expressed as (P.I.) and (A.I.) are presented in Table III along with NBT test results. PI was given by the number of candida positive cells per 100 neutrophils. PI increased in control immunized (P<0.001) group as well as in VHF lesion (P<0.001) and VHF sham (P<0.001) group animals when compared with controls, whereas the lesion and sham immunized group did not differ significantly from the control immunized group.

TABLE III : Neutrophil function test in control and experimental animals.

Group	Details	No. of animals	P.I.	A.I.	N.B.T
I	Control rats	15	76.1 ± 6.21	2.9 ± 0.72	10.73 ± 3.03
II	Control immunized	15	90.9 ± 3.35	3.28 ± 0.49	28.73 ± 6.17
III	Lesion	7	91.86 ± 3.13	3.05 ± 0.24	9.0 ± 3.61
IV	Lesion immunized	7	93.14 ± 2.67	3.18 ± 0.3	15.0 ± 4.2
V	Sham	7	94.29 ± 2.06	3.27 ± 0.18	9.14 ± 1.35
VI	Sham immunized	7	93.29 ± 3.99	3.37 ± 0.23	11.00 ± 2.94

The Values are expressed as mean ± SD

Our results indicates that VHF lesion has no influence on PI as the lesion and lesion immunized did not differ from sham and sham immunized group.

Avidity index or mean particle number was calculated by counting the number of candida particles within 100 candida positive neutrophils and taking the mean for single neutrophil. As none of the test group differ significantly from the controls, control immunized, sham and sham immunized group, we conclude that VHF lesion has no influence on neutrophil phagocytosis.

The spleen weight (mg)/body weight (gm) ratio and spleen cell count/spleen are given in Table IV and Fig. 1 respectively.

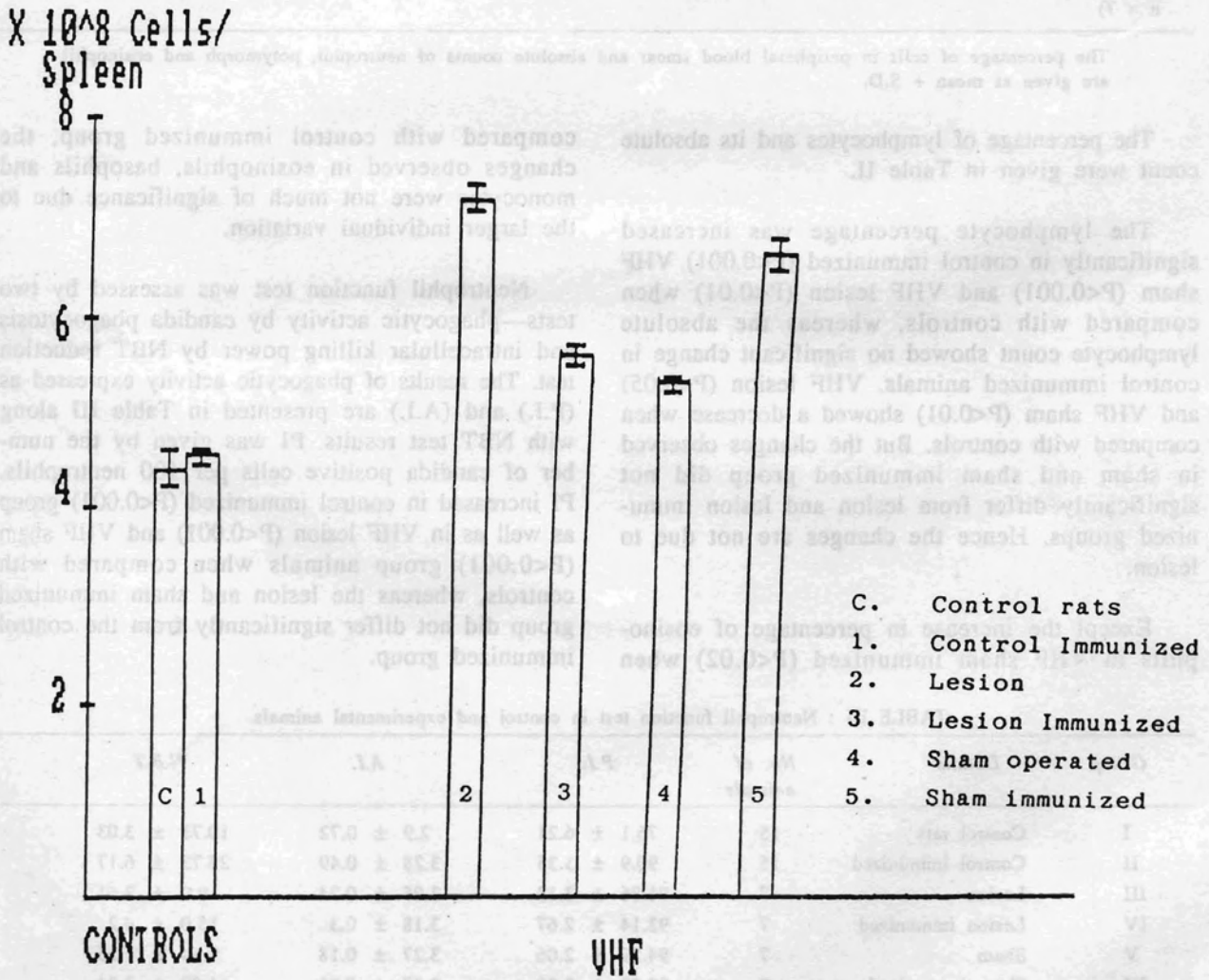

Fig. 1: Spleen Cell Count.

TABLE IV : Organ weight (mg)/body weight (g) ratio in control and experimental animals.

Group	*Details*	*No. of animals*	*Spleen wt/ body wt ratio*	*Thymus wt./ body wt ratio*	*Lymph node wt/ body wt. ratio*
I	Control rats	15	4.36±0.14	0.73±0.22	0.06±0.008
II	Control immunized	15	4.55±0.39	1.07±0.11	0.12±0.02
III	Lesion	7	4.42±0.69	0.62±0.9	0.09±0.02
IV	Lesion immunized	7	3.92±0.34	0.41±0.08	0.08±0.01
V	Sham	7	3.86±0.41	0.57±0.12	0.07±0.0078
VI	Sham immunized	7	5.01±1.21	0.58±0.15	0.11±0.03

The values are mean ± SD

The control immunized group did not differ significantly from control group in its organ weight ratio as well as in cell count.

The VHF sham immunized group showed a significant increase in weight ratio ($P<0.05$) as well as in cell count ($P<0.01$) when compared with its sham, whereas VHF lesion immunized showed a significant fall ($P<0.001$) in cell count without a significant change in weight ratio when compared with VHF lesion group.

The VHF sham group showed a significant decrease ($P<0.001$) in organ weight ratio without a significant alteration in cell count when compared with controls whereas the VHF lesion group showed a significant increase ($P<0.001$) in cell count without a significant change in organ weight ratio.

The VHF sham immunized group showed a significant increase ($P<0.05$) in organ weight ratio whereas the VHF lesion immunized group showed a significant fall ($P<0.01$) when compared with control immunized group. But both VHF sham immunized ($P<0.001$) and lesion immunized group ($P<0.001$) showed a significant increase in cell count.

In the VHF lesion group, the count was significantly increased ($P<0.001$) when compared with VHF sham group, showing the VHF incluences on the cell count. But VHF lesion immunized group showed a significant ($P<0.05$) decrease in spleen cell count when compared with its sham immunized group suggesting the role of VHF in modulating the spleen cell count.

Thymus weight (mgm)/body weight (gm) ratio and their counts are expressed in table IV and Fig. 2 respectively.

Thymus showed a significant increase in weight ratio ($P<0.001$) in control immunized group without a significant increase in cell count when compared with controls.

The VHF sham immunized showed a significant increase in cell count ($P<0.05$) with a significant increase ($P<0.02$) in its weight ratio when compared with the sham operated group, whereas the VHF lesion immunized showed a significant decrease in organ weight ratio ($P<0.001$) as well as in cell count ($P<0.01$) when compared with its lesion group.

The VHF lesion immunized and showed a significant fall ($P<0.01$) in thymic weight ratio while VHF lesion immunized alone showed a significant fall ($P<0.05$) in cell count when compared with control immunized group.

The VHF lesion immunized group showed a significant ($P<0.05$) fall in organ weight ratio and in cell count ($P<0.02$) when compared with its sham immunized group suggest that the specific anatomical site is important in maintaining organ weight and cell count during an immune response.

The popliteal lymph node weight (mgm)/body weight (gms) ratio is given in Table IV.

The lymph node weight ratio increased significantly in control immunized (P<0.001), VHF lesion (P<0.001) and VHF sham operated (P<0.02) when compared to the control group of rats. VHF sham immunized showed a significant increase in ratio (P<0.01) when compared with its sham group.

The VHF lesion immunized showed a significant decrease (P<0.001) in the ratio when compared with control immunized animals.

Since the VHF lesion is not significantly different from sham and the sham immunized group is similar to lesion immunized group we conclude that VHF has no role in maintaining lymphnode weight ratio.

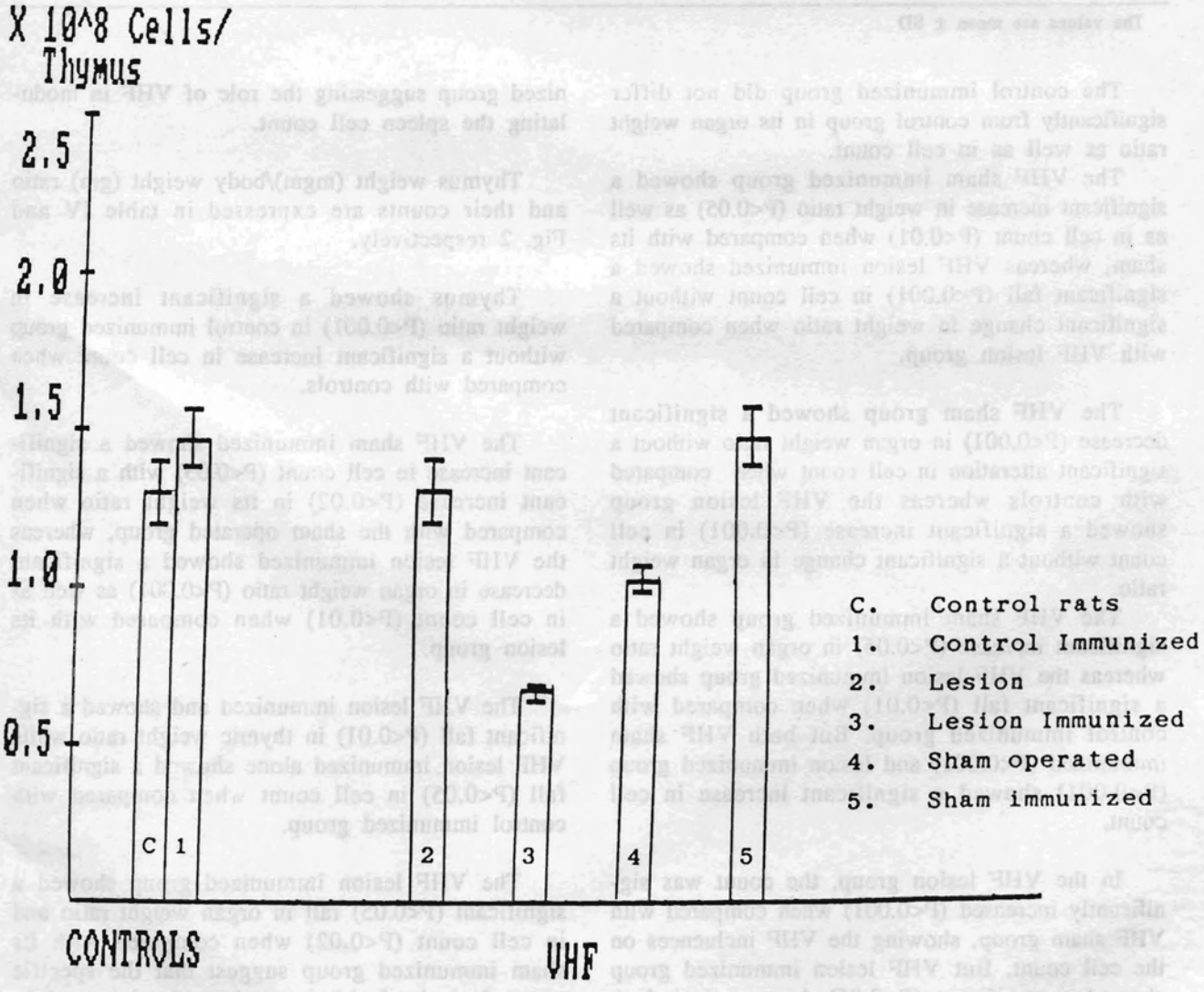

Fig. 2: Thymus Cell Count.

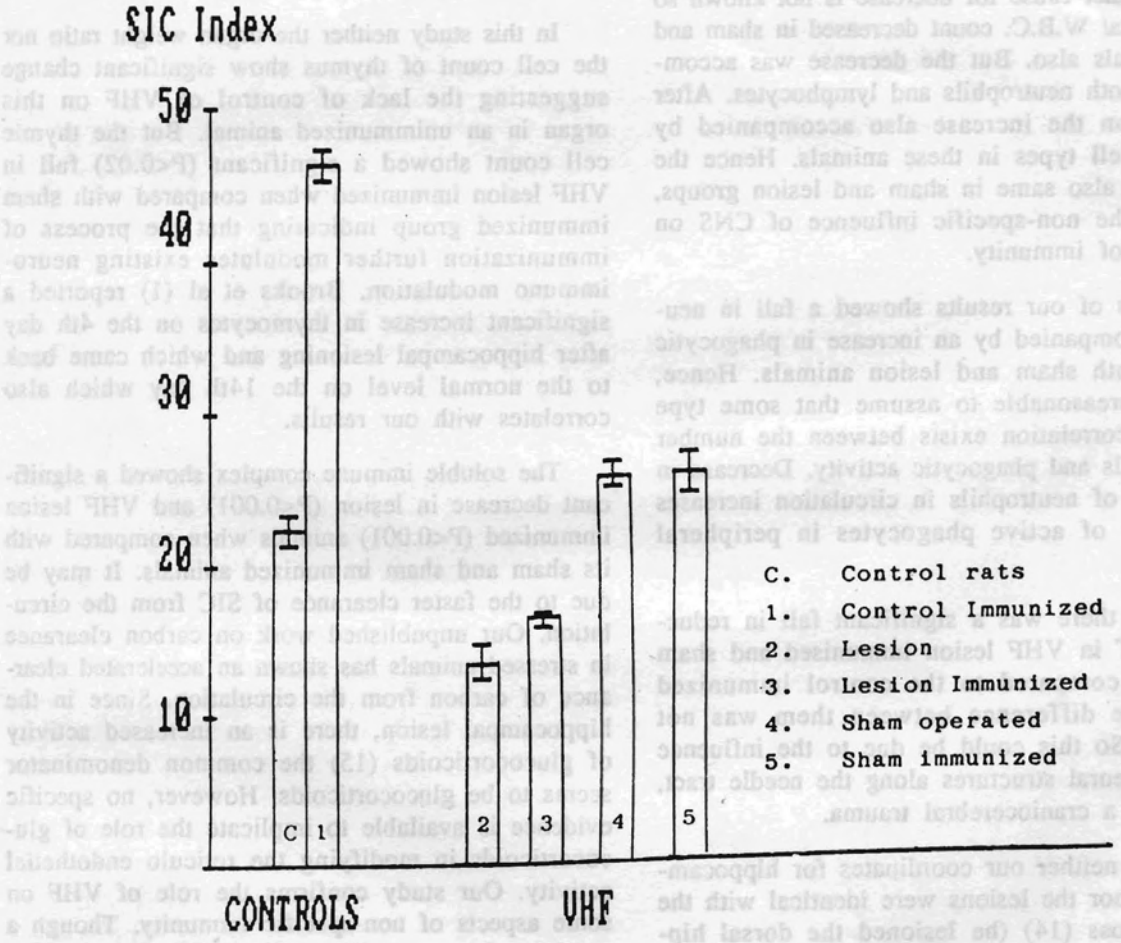

Fig. 3: Souble Immune Complex.

The soluble immune complex (SIC) Index is given in Fig. 3.

SIC index showed a significant increase ($P<0.001$) in control immunized group and a significant fall in VHF lesion ($P<0.02$) group when compared with controls.

SIC index showed a significant ($P<0.001$) fall in VHF lesion and VHF lesion immunized ($P<0.01$) group when compared with VHF sham and sham immunized group. This suggests that VHF can influence kinetics of SIC.

DISCUSSION

To determine the influence of VHF on the immune system the results were compared in all possible combinations to get a clear conclusion.

All the groups including control immunized group showed a significant fall in Total W.B.C. count when compared with controls. It has been shown that leucopenia is characteristically seen when foreign proteins are parenterally introduced (13). The exact cause for decrease is not known so far. The Total W.B.C. count decreased in sham and lesion animals also. But the decrease was accompanied by both neutrophils and lymphocytes. After immunization the increase also accompanied by these two cell types in these animals. Hence the pattern was also same in sham and lesion groups, indicating the non-specific influence of CNS on this aspect of immunity.

Analysis of our results showed a fall in neutrophils accompanied by an increase in phagocytic Index in both sham and lesion animals. Hence, it is not unreasonable to assume that some type of inverse correlation exists between the number of neutrophils and phagocytic activity. Decrease in the number of neutrophils in circulation increases the number of active phagocytes in peripheral blood.

Though there was a significant fall in reduction of NBT in VHF lesion immunised and sham immunized compared to the control immunized animals, the difference between them was not significant. So this could be due to the influence of certain neural structures along the needle tract, resulting in a craniocerebral trauma.

Though neither our coordinates for hippocampal lesions nor the lesions were identical with the work of Cross (14) (he lesioned the dorsal hippocampus), our study also shows hyper cellularity in spleen of VHF lesioned animals. This indicates that not only dorsal hippocampus but also the ventral hippocampus can influence splenic cellularity. The increase in spleen cell count in lesioned animals compared to sham operated animals indicates the specific lesion effect of VHF. Immunization of the lesioned animals on the other hand decreases the spleen cell count significantly indicating thereby that the immunization process modulates the specific lesion effect. It is not known what type of cell increases in the spleen of test animals.

In this study neither the organ weight ratio nor the cell count of thymus show significant change suggesting the lack of control of VHF on this organ in an unimmunized animal. But the thymic cell count showed a significant ($P<0.02$) fall in VHF lesion immunized when compared with sham immunized group indicating that the process of immunization further modulates existing neuro-immuno modulation. Brooks et al (1) reported a significant increase in thymocytes on the 4th day after hippocampal lesioning and which came back to the normal level on the 14th day which also correlates with our results.

The soluble immune complex showed a significant decrease in lesion ($P<0.001$) and VHF lesion immunized ($P<0.001$) animals when compared with its sham and sham immunized animals. It may be due to the faster clearance of SIC from the circulation. Our unpublished work on carbon clearance in stressed animals has shown an accelerated clearance of carbon from the circulation. Since in the hippocampal lesion, there is an increased activity of glucocorticoids (15) the common denominator seems to be glucocorticoids. However, no specific evidence is available to implicate the role of glucocorticoids in modifying the reticulo endothelial activity. Our study confirms the role of VHF on some aspects of non-specific immunity. Though a number of non-specific and specific lesion effect has come to light, the direct link between the two systems is yet to be worked out.

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