Original Research Article

Effect of bilateral cerebellar fastigial nucleus lesion on non-specific immune responses in adult Wistar albino rats

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Abstract

Background: The field of neuroimmunomodulation is gaining attention among researchers and scientists as it helps to focus new pathophysiological concepts behind the allergic, autoimmune and infectious disorders. Aim: The aim of the present study was to assess the effect of bilateral cerebellar fastigial nucleus lesion on non-specific immune responses in adult male Wistar albino rats. **Materials and Methods:** Healthy adult male Wistar albino rats weighing about 200-220 gm were chosen for our study and were subjected to bilateral cerebellar fastigial nucleus lesion. The study design consists of three groups namely Group I: Control immunized animals; Group II: Bilateral fastigial nucleus lesioned immunized animals; Group III: Sham operated immunized animals. The animals were immunized on 10th day by injecting 1 ml of the sheep red blood cell (SRBC) suspension intra-peritoneally and on the 15th day, blood samples were collected to test immunological parameters namely plasma corticosterone levels, total leucocyte count, differential leucocyte count, splenic and thymic cell counts. **Results:** In our study, there was a significant decrease in both splenic and thymic cell counts in lesion immunized animals and a marked increase in the neutrophil count with a concomitant decrease in the lymphocyte count also. The total leucocyte count and plasma corticosterone levels did not vary from its respective control groups. **Conclusion:** This study further substantiates the bi-directional cross talk communication existing between the neuro-endocrine and immune system

Key words: cerebellum, immune responses

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Introduction

The emerging recent advances in the field of neuroimmunomodulation have shown that the nervous system and the immune system are closely inter-linked.[1,2] The role of different brain areas like cerebral cortex, hypothalamus, basal ganglia, etc. in immunomodulation has been reported by several investigators.[3-6] It offers a new insight and a better understanding of stress disorders, affective disorders and dementia. For decades the role of the cerebellum has been thought of as being confined to controlling voluntary motor activities. The cerebellum is a part of the hind brain that serves as a regulator of voluntary motor activity and a relay station for unconscious proprioception and balance.[7,8] Besides its well known role in voluntary motor activities, the cerebellum has been found to be involved in the regulation of the activities of the cardiovascular, respiratory and gastrointestinal systems.[9-14] Very few studies have been done on the role of cerebellar fastigial nucleus lesion and its effect on immune responses.

The neural areas of brain regulating the activity of autonomic nervous system are potential candidates as a centre of immunomodulation because neural areas regulate immune system by sympathetic activity and hypothalamo-pituitary-adrenal (HPA) axis. Since there are no direct

connections existing between the cerebellum and the immune system, some studies have reported cerebellar influence on immune function via pathways that are largely unknown. Some observations have implied a cerebellar influence on immune functions via direct bi-directional pathways between the cerebellum and the hypothalamus namely, cerebello-hypothalamic projections and hypothalamo cerebellar projections.[13-14]

Previous studies have reported that kainic acid induced lesion of the vestibulocerebellum in rats caused immunosuppressive effects.[15] In one of the earlier reports, bilateral fastigial nucleus lesions resulted in T lymphocyte proliferation and the NK cell cytotoxicity, suggesting that the spinocerebellum participated in the modulation of lymphocyte functions. [16]Our previous study has reported that cerebellar fastigial nucleus lesion influences the cell mediated as well as the humoral immune responses in rats.[17]

Based on this, our study has been designed to understand the role of the cerebellar fastigial nucleus lesion in modulating the immune cells and immune responses.

Materials and methods:

This study was approved by the Institute's Animal Ethical Committee (IAEC No. 08/034/07) and the Committee for the Purpose of Control and Supervision of Experiments on Animals, University of Madras, Chennai, India and was conducted in Dr.ALM PG Institute of Basic Medical Sciences, Taramani, Chennai. Healthy adult male Wistar albino rats weighing about 200-220 g have been used for this study and allowed to have food and water *ad libitum*. The animals were maintained in appropriate environmental conditions of temperature and humidity on an alternative 12-hour light/dark cycle.

Since the interested site of lesion is not on the surface, inevitably the cortical structures above are also destroyed during the surgical procedure and it has been reported that these structures when destroyed modulate the immune responses.[18] Hence, sham animals are considered as the strict control to evaluate the lesion effect. To avoid variations in the measured results due to circadian rhythm, stress free blood samples collections were done between 8:00-10:00 am.[19] Animals were divided into three groups with six animals in each group as follows:

Group I: Control immunized animals,
Group II: Bilateral fastigial nucleus lesioned immunized animals and
Group III: Sham operated immunized animals.

Fastigial nucleus lesion: Rats were anaesthetized with Pentothal Sodium (40 mg/kg body weight). The hair on the scalp was removed and the animal was fixed to the stereotaxic apparatus. The coordinates for fastigial nucleus is minus 10 mm from Bregma, 1.10 mm lateral from the midline, 4.80 mm from dura (depth).[20] Appropriate holes were made and using stainless steel electrode of 0.22 mm diameter, anodal electric lesions were made on both sides of the cerebellum with direct current of 2mA at 100 volts for 10 seconds. The lesioned as well as the sham lesioned animals were allowed to recover for 10 days postoperatively and then were subjected to immune studies on the 15thday.

Immunization protocol: Sheep red blood cells (SRBC) were collected in sterile Alsever's solution and washed three times with pyrogen free normal saline and then adjusted to 5x10^{9}cells/ml saline. On the 10thday after the surgery, the animals were then immunized by intra-peritoneal injection of 1 ml of the SRBC suspension. On the 15thday all the immunological parameters were studied.

Plasma corticosterone estimation: To1 ml of plasma, purified dichloromethane (7.5 ml) was added and gently shaken for 5 min. To the sediment (supernatant discarded), fluorescence reagent (2.5 ml) ethanol and concentrated H2SO4 in the ratio 3:7 was added and shaken vigorously for 20 s. The resulting fluorescence of the acid layer was read at excitation 470 nm and emission 530 nm in fluorescence spectrophotometer.[21]

Total WBC count and differential count: Blood samples were diluted with Turk's fluid in a WBC pipette and counted in an improved Neubauer counting chamber under a low power magnification. Differential count smears were made from the freshly drawn blood specimens and stained with Leishman's stain and were preserved for counting.[15]

Organ cell counts: The spleen and thymus were removed, blotted and weighed. They are placed in minimum essential medium (SIGMA M0894) and teased to give a cell suspension. After centrifugation

and washing, the cells were made up to a known volume and the number of nucleated cells was determined by counting using a haemocytometer and Turks fluid (WBC diluting fluid). The results are expressed as x 10^8 cells[22].

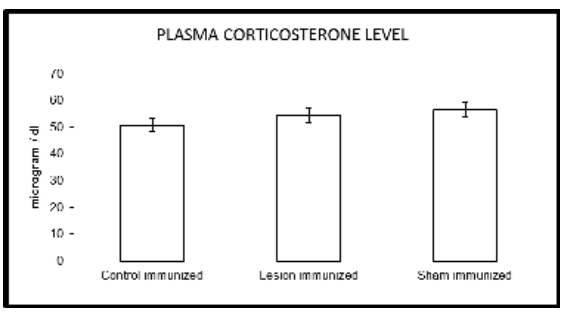
Statistical Analysis: All the data were statistically analysed using one way ANOVA followed by Tukey's multiple comparison tests. $p< 0.05$ was considered significant.

Results

For understanding the effect of the lesion, the sham group was considered as a strict control and was compared with controls. All the animals were healthy and no significant weight loss was observed in any of the groups.

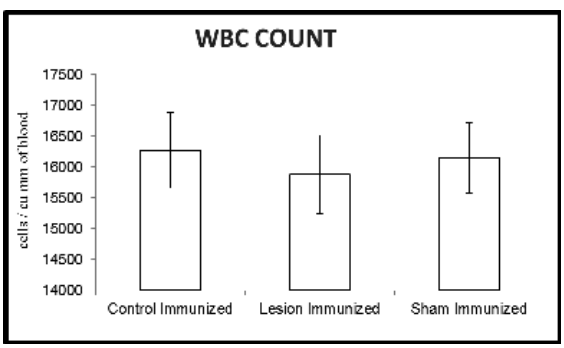
Plasma corticosterone levels: The data from all groups are shown in the bar diagram (Figure 1) as Mean ± SD. The bilateral lesioned immunized groups did not show significant changes from the immunized control and sham immunized animals.

Figure1: Plasma corticosterone levels of Wistar albino rats



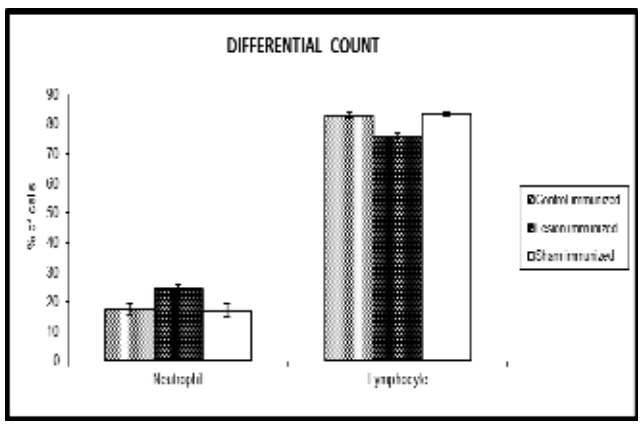
Total WBC count: The data from all groups expressed as Mean ± SD are shown in the bar diagram (Figure 2). There was no significant change observed in lesion immunized animals when compared with the control immunized and sham operated immunized animals.

Figure 2: Total WBC count of Wistar albino rats



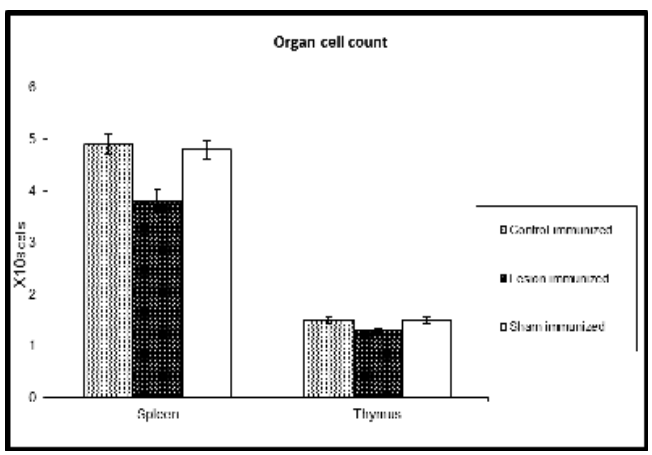
Differential count of Neutrophils and lymphocytes: The data from all groups are shown in the bar diagram (Figure 3) with Mean ± SD. The percentage of neutrophils in bilateral lesioned immunized animals showed a significant increase from immunized control as well as from the respective sham immunized animals. The percentage of lymphocyte in bilateral lesioned immunized animals showed a marked decrease from control as well as from sham immunized animals.

Figure 3: Differential count of Neutrophils and lymphocytes of Wistar albino rats



Organ cell counts (spleen and thymus): The data from all groups are shown in the bar diagram (Figure 4) with mean ± SD. Spleen cell count of bilateral lesioned immunized groups has shown a significant decrease when compared to immunized control and respective immunized sham operated groups. Thymus cell count in bilateral fastigial lesioned immunized animals showed a significant decrease from the immunized control and their respective immunized sham groups.

Figure 4: Organ cell counts (spleen and thymus) of Wistar albino rats



Discussion

The results of our study further confirm the role of cerebellum in immunomodulation. In this study, care has been taken to have consistent reproducible results and therefore electrolytic lesions were preferred. Hippocampal damage in monkeys result in selective alteration of the HPA axis function and there was an increase in plasma corticosterone levels which is in contrast to our study (where there was no significant change in plasma corticosterone levels).[23] It has been shown that leucopenia is characteristically seen when foreign proteins are parenternally introduced.[24] In our study there was no change in total WBC count which was contradictory to the findings of earlier studies.[25] Circulation of immune cells is essential for maintaining an effective immune defense network. [26] There was a decrease in the lymphocyte population with increase in neutrophil percentage in our study which indicates the inverse relation existing between the lymphocytes and neutrophils.[27]

After immunization, there was a marked decrease in thymus cell count observed in bilateral lesioned animals. Such decrease in splenic cell count as well as thymus cell count was observed after bilateral ventral hippocampal formation lesioned animals.[28] Based on these reports it can be concluded that the fastigial nucleus lesion induced alteration could be modulated through hypothalamus. The nervous system can communicate with the immune system via HPA axis by glucocorticoids to the periphery[29] or by sympathetic activation.[30] Since in our study the plasma corticosterone levels did not show any variation, the influence of HPA axis on immune responses could not be considered and further exploration of the functional pathways mediating these changes need to be observed.

Conclusion

The results of our study further substantiate the bi-directional cross talk communication existing between the neuro-endocrine and immune system

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Conflicts of interest: Nil

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