European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 4; Issue 9; September 2016; Page No. 38-41

Basolateral amygdala influences the phagocytosis of reticuloendothelial cells in rats

[1] Nilotpal Mandal, [2] Ananda Raj Goswami, [3] Goutam Dutta, [4] Durjoy Majumder, [*5] Tusharkanti Ghosh

[1] Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India
[1, 2, 3, 5] Neurophysiology Laboratory, Department of Physiology, University College of Science & Technology, University of Calcutta, Kolkata, West Bengal, India
[4] Department of Physiology, West Bengal State University, 24 Pgs (N), Barasat, Kolkata, West Bengal, India

Abstract
The basolateral amygdala (BLA) appears to be a potential brain area for regulating immune responses when its neuroanatomical connections and functions are considered. The phagocytic activity of reticuloendothelial (RE) cells, a part of the innate immune responses, was used to assess the immunomodulatory effects of different extracts from plants and animals. In an attempt to study the immunomodulatory role of BLA on the phagocytic activity of RE cells, BLA was lesioned electrolytically in rats and the phagocytic activity of RE cells was assessed by carbon clearance test after two weeks of surgery along with the measurement of serum corticosterone level. The phagocytic activity of RE cells and serum corticosterone level were decreased in the BLA lesioned rats compared to that of control and sham operated rats. This study shows that the phagocytic activity of RE cells is suppressed in BLA lesioned rats which probably indicates a stimulatory influence of BLA on the observed innate immune response in intact control animals.

Keywords: BLA, RE cells, phagocytic activity, corticosterone

1. Introduction
The amygdala is well known for its role in emotional experience [1] and emotional memory in humans [2]. The autonomic changes associated with emotion may be related to the activity of amygdala as it has been reported that amygdala has direct neural connections to the autonomic preganglionic neurons, hypothalamus and other neural areas regulating autonomic functions [3]. The immune responses are altered with the emotional status of humans [4-6]. In experimental animals different CNS areas involved with the regulation of emotion and autonomic functions were reported to influence immune responses [7].

Though the hypothalamic nuclei such as paraventricular nucleus (PVN), anterior hypothalamus (AH), medial hypothalamus (MH) were investigated extensively to assess their participation on the immune responses in animals [8-11], the involvement of amygdala in regulating immune responses has been investigated very little. Some investigators have reported that lesion in amygdaloid complex and hippocampus in rats led to enhanced proliferative response of spleen cells and thymocytes to Con a stimulation [12-14].

The amygdaloid complex consists of many nuclei such as basolateral, medial, central and ventromedial [15]. The basolateral amygdala (BLA) was reported to be related with memory consolidation and activity behavior [16-18]. The rats became hypoactive after lesion of BLA but did not show any change of NKCC (Natural killer cell cytotoxicity) activity [19].

The phagocytic activity has been used by several investigators to study the immunomodulatory roles of different brain areas such as hippocampus and hypothalamus [11, 20, 21]. The phagocytic activity of RE cells has been measured *in vivo* by carbon clearance test [22-26] and was used as indicator to assess the immunomodulatory effects of plant and animal extracts [23-27]. The serum corticosterone in rats is important for influencing the immune responses and has been measured by several investigators along with immune responses in animals and humans to identify the regulating factors for the changes of immune responses [28-31]. In the present study the phagocytic activity of RE cells and serum corticosterone level have been measured by carbon clearance test in BLA lesioned rats in an attempt to study the immunomodulatory role of BLA in rats.

2. Method
Animals
In this study 36 male albino rats (Charles-Foster strain) weighing 200–220g were used. Animals were housed individually in polypropylene animal cages with food pellets and water ad libitum in the animal room with a 12-hour light-dark cycle (light 7 a.m. to 7 p.m.). The animal room was maintained at a temperature of 25 ±1 °C. Adequate measures were taken to minimize the pain and discomfort of the rats as per institutional ethical regulation.

Design of Experiments
Experiment I
(Phagocytic activity of RE cells): Eighteen rats were equally divided into 3 groups: control, sham-operated group (electrode was inserted into BLA without electrolytic coagulation) and basolateral amygdala (BLA) lesioned group (BLA was coagulated electrolytically). The phagocytic activity of reticuloendothelial cells (RE cells) was assayed by carbon clearance test in BLA lesioned rats after two weeks of lesion along with control and sham-operated rats.

Experiment II
(Serum Corticosterone Concentration): Eighteen rats were equally divided into 3 groups: control, sham-operated and BLA-lesioned groups. The serum corticosterone concentration

was measured by RIA in BLA lesioned rats after 2 weeks of surgery along with the control and sham-operated groups.

Lesion of Basolateral Amygdala

A nichrome-coated bipolar electrode (tip was uninsulated) was placed stereotaxically at 2mm posterior to the bregma, ±4.5mm lateral to midline and 8mm below the skull surface with bregma and lambda in the same horizontal plane. Na-thiopental (50 mg/kg body weight, i.p.) was used as anesthesia. 2 mA DC current were passed for 10 second through the bipolar electrode to make a lesion in Amygdala lesioned (BLA).

Carbon Clearance Test

The RE cell phagocytic activity was measured by the methods of carbon clearance test as described by others [22, 27]. A carbon suspension was obtained from carbon ink (India ink, Camal) which contained approximately 100 mg carbon/ ml, suspended in a fish glue solution. It was centrifuged at 500 rpm for 15 min and the carbon was obtained from the precipitate. 10mg of carbon was dissolved in 100 ml distilled water. After deep anesthesia with sodium thiopental (50 mg/kg body wt.), 0.1 ml carbon suspension (10%) was injected into the tail vein of the rat and 20µl of blood was collected from retro-orbital sinus at the interval of 5 min into three 5 ml test tubes. 20µl of blood was also collected from retro-orbital sinus before the injection of carbon suspension in each rat. Thus four samples of blood were collected at 0, 5, 10 and 15 min after carbon suspension injection in each rat. 2ml of acetic acid (1%) was added with each 20µl of blood sample and the absorbance of mixed solution was measured at 660 nm by spectrophotometer (Simadzu, Zapan). A regression line was drawn from the mean absorbance values at different time intervals for each group of rats (MINITAB statistical software). This slope of the regression line was considered as the phagocytic index (PI) of RE cells.

Serum Corticosterone Concentration

The blood was collected (1ml without anticoagulant) from the heart of a deeply anesthetized rat (Na-thiopental, 50 mg/kg body weight, i.p.) by a syringe between 2: 30 and 3: 00 p.m. on the day of sacrifice. The serum from the blood was used for measuring corticosterone. The concentration of the collected serum was determined by radioimmunoassay using a commercially available kit [125] Rat Corticosterone (Coat-A-Count, Siemens Healthcare Diagnostic Inc, USA)]. The antisera used for the assay was highly specific for the rat corticosterone and it had 1.58% cross-reactivity with 11-deoxy corticosterone. The assay sensitivity was approximately 5.7 µg/dl and intra-/inter-assay coefficient variation is < 6%.

Confirmation of Lesion by Histology

The BLA lesioned rats of Experiment I and II were sacrificed after 2 weeks of surgery by deep anesthesia (Na-thiopental, 50 mg/kg body weight, i.p.) and the brain was perfused intracardially with 0.9% saline followed by 10% formaldehyde solution. The brains were removed from the skulls and were kept in 4% formaldehyde solution for fixation. After dehydration and clearing, paraffin blocks of those brains were prepared and 10 µm thick sections were cut by a microtome. The brain sections were stained by hematoxylin-eosin to identify the lesioned area (Fig. 1).

Statistical Analysis

Data are expressed as mean ± SEM. One-way ANOVA was employed to compare the data of the control, sham-operated and BLA-lesioned groups followed by LSD post hoc test using the Statistical Package for Social Science Software (SPSS software: 9.0.0, USA). $P \leq 0.05$ was considered as a significant difference.

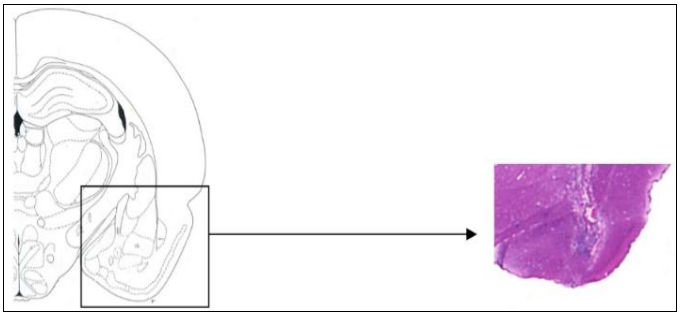

Fig 1: Hematoxylin-eosin-stained histological section of rat brain showing the BLA-lesioned area along with its location in a coronal section.

3. Results

Phagocytic activity of RE cells

The phagocytic activity of RE cells were decreased as indicated by increased phagocytic index ($p<0.01$) in the BLA lesioned rats compared to that of control and sham-operated rats (Fig. 2). The phagocytic index measured from the rate of carbon clearance in BLA lesioned rats was - 0.0006±0.0004. The mean phagocytic indices of control and sham operated groups were - 0.004±0.0001 and - 0.002±0.0003 respectively.

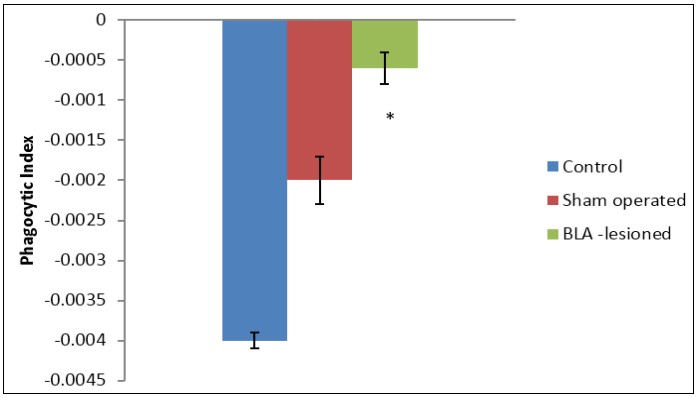

Fig 2: The PI of RE cell in the control, sham-operated and basolateral amygdala (BLA) lesioned rats after 2 weeks of surgery. * Significant at $P < 0.01$, between BLA lesioned rats and control/sham-operated rats. The values are expressed as mean ± SEM (n = 6).

Serum Corticosterone Concentration

The serum corticosterone concentration in BLA lesioned rats was significantly decreased compared to that in the control [$F(2, 9) = 12.12$, $P < 0.05$] and sham-operated rats [$F(2, 9) = 12.12$, $P < 0.01$]. (Table 1).

Table1: Serum corticosterone concentration in the control, sham-operated and basolateral amygdala (BLA) lesioned rats after 2 weeks of surgery.

Groups	Serum corticosterone concentration (µg/dl)
Control	174.1±10.033
Sham-operated	180.2±7.076
Basolateral Amygdala (BLA) lesioned	90.7±12.186*,+

Values are expressed as mean ±SEM, n= 6 * Significant at $p < 0.01$ between BLA lesioned rats and control groups, +$p < 0.05$ between BLA lesioned rats and sham operated groups.

4. Discussion

In the present study the effects of the lesion of basolateral amygdala (BLA) on phagocytic activity of RE cells by carbon clearance test have been studied. The RE cells include sessile and fixed macrophages, Kupffer's cells and other free histiocytes such as monocytes, neutrophil etc [26]. These RE cells act as first line of defense in the body by its ability to scavenge debris, bacteria and other foreign matter by phagocytic activity [23, 32]. These cells also help in the defense mechanism by secreting cytokines such as IL1, IL6, TNF α and reactive oxygen species [25, 32]. RE cells have the ability to remove carbon particle and silicon by ingesting them through phagocytosis. The phagocytic activity of RE cell has been studied by several investigators using carbon clearance test to assess the immunomodulatory effects of several plant and animal extracts [23-27].

The phagocytic activity as an innate defense mechanism has been used by several investigators to explore the immunomodulatory role of different areas of central nervous system [11, 20]. The phagocytic activity of blood WBC was increased after lesion of medial septum [34], posterior cerebellum [35], and electrical stimulation of hippocampus [11] while it was inhibited after lesion of paraventricular nucleus (PVN) of hypothalamus [20]. The phgocytic activity of RE cells have not been assessed to explore the immunomodulatory role of different CNS areas. In the present study RE cells showed decreased phagocytic activity in carbon clearance test in BLA lesioned rats. Though any behavioral test was not done in BLA lesioned rats of the present study, others have reported hypoactivity in novelty test in BLA lesion rats [19]. This behavioral change may be associated with the changes of some immune responses as HPA axis and cytokines are changed with behavioral pattern [36]. However, NKCC activity was not changed in BLA lesioned rats when the hypoactivity was noted [19]. The present study showed that RE cells activity were changed in BLA lesioned rats indicating an immunomodulatory role of BLA on the one component of innate immune responses. The involvement of amygdaloid complex in immune regulation was indicated by the observation that the number of thymocytes and spleen cells were increased and proliferative response of these cells to Con A were enhanced after lesion of amygdaloid complex in rats [12-14]. Though cytokines in BLA lesioned rats were not measured in the present study the activity of HPA axis was assessed by measuring serum corticosterone. The plasma corticosterone level was decreased significantly after 2 weeks of lesion of BLA which can be supported by the observation of Gafford *et al.* 1981 [37] who reported that the adrenocortical responses as well as the glucocorticoid release were inhibited after lesion of amygdala. This lower corticosterone cannot explain the decreased phagocytic activity of RE cells as the phagocytic activity of macrophages were inhibited by higher dose of glucocorticoids [28, 38]. The effect of excess in vitro glucocorticoids on the phagocytic activity of macrophages was investigated and it was reported that the phagocytic activity of macrophages was suppressed in a high dose of glucocorticoids but was increased in a low dose [28, 38]. The in vivo phagocytic activity of macrophages is influenced by many signals such as cytokines including corticosterone [29, 30]. There are only few studies on the effect of in vivo glucocorticoids on the macrophage functions [31, 39]. Though some investigators reported a biphasic effects of glucocorticoids on inflammatory responses similar to that of in vitro study [29, 31], others found that the inflammatory response of monocytes did not enhance in human subjects after depletion of endogenous glucocorticoids by oral RU486 (blocker of intracellular glucocorticoids receptors) and intravenous (i.v) administration of etomidate at a low dose that inhibit adrenal 11-β hydoxylase [39]. The later investigators suggest the in vivo effect of glucocorticoids is not universally anti-inflammatory as the depletion of glucocorticoids fails to increase the proinflammatory responsiveness of monocytes. The low serum level of corticosterone with a concomitant lower phagocytic activity in BLA lesioned rats may therefore be reconciled from observation of [39] but the mechanism of this change of phagocytic activity of RE cells in BLA lesioned rats cannot be ascertained from this experiment. However, this study shows that the phagocytic activity of RE cells was suppressed in rats after 2 weeks of BLA lesion probably indicating a stimulatory influence of this nucleus on the phagocytic activity of RE cells in intact control animals.

5. References

1. Morris JS, Frilt CD, Perrett DI, Rowland D, Yong AN, Calder AJ, *et al.* A different neural response in human amygdala is fearful and happy facial expressions. Nature 1996; 383:812-815.
2. Kandel ER, Schwartz JH, Jessell TM. Principles of neural science. Mc Graw-Hill, New Yark. 2000, 988-989.
3. Ader R, Madden K, Felten DL, Bellinger DL, Schiffer RB. Interactions between the brain and the immune system, psychoneuroimmunology. In: Fogel BS, Schiffer RB, (Eds.): Willams Wilkins, Philadelphia: Neuropsychiatry, 1996, pp. 611-646.
4. Schleifer SJ, Keller SE,Camerino M, Thornton JC, Stein M. Suppression of lymphocyte stimulation following bereavement. *JAMA.* 1983; 250:374-377.
5. Irwin M, Daniels M, Smith TL, Bloom E, Weiner H. Impaired natural killer cell activity during bereavement. Brain Behav Immu. 1987; 1:98-104.
6. Kiecolt-glaser JK, Glaser R. Psychological influences on immunity. *Psychosomatics*. 1986; 27:621-624.
7. Wrona D. Neural immune interaction: an integrative view of the bidirectional relationship between the brain and immune systems. J Neuroimmunol. 2006; 172:38-58.
8. Felten DL, Ader R, Cohen N, Felten SY, Carlson SL,

Roszman TL. Central neural circuits involved in neural-immune interactions. In Ader R, Felten DL, Cohen N, (Eds.): Psychoneuroimmunology-II. Academic press, New York, 1991, pp. 3-25.
9. Forni G, Bindoni M, Santoni A, Belluardo N, Marchese AE, Giovarelli M. Radio frequency destruction of tubero infundibular region of the hypothalamus permeability abrogates NK cell activity in mice. Nature. 1983; 306(5939):10-16
10. Baciu I, Hriscu M, Saulea G. Hypothamic mechanism of imunity. *Int.J Neurosci*. 2003; 113:259-277.
11. Hefco V, Olariu A, Neacsu I, Isaicul A. The ways throμgh which the hypothalamic paraventricular nucleus (PVN) and the medial hypothalamus affect the organisms defense function. Rom J Physiol. 1993; 30:87-91.
12. Cross RJ, Brooks WH, Roszman TL, Markesbery WR. Hypothalamic immune interactions. Effect of hypophysectomy on neuroimmunomodulation. J Neurol Sci. 1982; 53:557-566.
13. Pan, Q, Long J. Lesion of hippocampus enhance or depress humoral immunity in rats. Neuroreport. 1993; 4:864-866.
14. Brooks WH, Cross RJ, Roszman TL, Markes Bery WR, Nuroimmunemodulation. Neural anatomical basis for impairment and facilitation. Ann. Neurol. 1982; 12:56-61.
15. Ledoux, J.E., Farb, C, and Romanski, L.M. Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neurosci Lett.* 2007; 134: 139–144.
16. Canteras NS, Swanson LW. Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. J Comp Neurol.1992; 324:180-194.
17. Ledoux JE, Farb C, Rμggioro DA. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J Neurosci. 1990; 10:1043-1054.
18. Maren S. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. Trends Neurosci. 1999; 22:561-567.
19. Grijalva CV, Levin ED, Morgan M, Roland B, Martin FC. Contrasting effects of centromedial and basolateral amygdaloid lesions on stress-related responses in the rat. Physiology behaviour. 1990; 48:495-500.
20. Hefco V, Olariu A, Neacsu I, Isaicul A. The ways throμgh which the hypothalamic paraventricular nucleus (PVN) and the medial hypothalamus affect the organisms defense function. Rom J Physiol. 1993; 30:87-91.
21. Hefco V, Yamada K, Hefco A, Hritcu L, Tiron A, Nabeshima T. The interaction between the cholinergic and dopaminergic system in learning and memory process in rats. Rom J Physiol. 2004; 41(1-2):21-30.
22. Biozzi G, Benacerraf B, Halperm BN. Effet de la vaccination par *Plasmodium berghei* irradié sur l'activité phagocytaire du système réticuloendothélial au cours de l'infection du rat par ce plasmodium. Bull World Health Organ. 1970; 42(1):163-168.
23. Benmebarek A, Zerizer S, Laggoune S, Kabouche Z. Immunostimulatory activity of Stachys mialhesi de Noé. Allergy, Asthma & Clinical Immunology. 2013; 9(2):1-4.
24. Pattanayak SP, Mazumder PM. Immunomodulatory Activities of *Dendrophthoe falcata* (L.f) Ettingsh in Experimental Animals: *In vitro* and *In vivo* Investigations. Journal ofscientific research.2011; 3(3):619-630.
25. Aribi B, Zerizer S, Kabouche Z. Immunomodulatory Activity of Argania Spinosa Seeds. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(3):488-491.
26. Tripathi S, Maurya A, Kahrana M, Kaul A, Sahu RK. Immunomodulatory property of ethanolic extract of *Trigonella Foenum-Graeceum* leaves on mice. Der Pharmacia Lettre, 2012; 4(2):708-713.
27. Ponkshe CA, Indap MM. *In vivo* and *In vitro* evaluatori for immunomodulatory activity of three marine animal extracts with reference to phagocytosis. Ind.J. Expt.Biology. 2002; 40:1399-1402.
28. Zhong HJ, Wang HY, Yang C, Zhou JY, Xin J. Jiang low concentrations of corticosterone exert stimulatory effects on macrophage function in a manner dependent on glucocorticoid receptors. Int J Endocrinol. 2013; 405-127.
29. Liao J, Keiser JA, Scales WE, Kunkel SL, Kluger MJ. Role of corticosterone in TNF and IL-6 production in isolated perfused rat liver. Am J Physiol. 1995; 268(3):699-706.
30. Petrovsky N, Socha L, Silva D, Grossman AB, Metz C, Bucala R. Macrophage migration inhibitory factor exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator. Immunology and Cell Biology. 2003; 81:137-143.
31. Lim HY, Müller N, Herold MJ, Brandt JVD, Reichardt HM. Glucocorticoids exert opposing effects on macrophage function dependent on their concentration. Immunology. 2007; 122: 47-53.
32. Sigurd J, Normann MD, Earl P, Benditt MD. Function of the reticuloendothelial System. Seattle: Department of Pathology, University of Washingt. 1965; 693-707.
33. Sembulingam K, Sembulingam P. Essentials of medical physiology. Jaypee, New Delhi. 2006; 130-131.
34. Dutta G, Mandal N, Goswami A, Majumdar D, Ghosh TK. Effects of Electrolytic Lesion of Medial Septum on Some Immune Responses in Rats. Neuroimmunomodulation. 2011; 18: 232-239.
35. Goswami AR, Dutta G, Ghosh TK. Effects of lesion of posterior part of cerebeller varmis and fastigial nuclei on some immune responses in male rats. Asian journal of biomedical and pharmacutical sciences. 2014; 04(37):38-43.
36. Gao Y, Huang Y, Lin J, Wang D, Lin R. Areas of brain involved in immunoregulation. Zhongguoyi Xue Yuan Xue Bao. 2000; 22:525-528.
37. Gafford GM, Ressler KJ . GABA and NMDA receptors in CRF neurons have opposing effects in fear acquisition and anxiety in centralamygdala vs. bed nucleus of the stria terminalis. Horm Behav. 2015; 76: 136-42.
38. Goulding NJ, Flower RJ: Glucocorticoids and the immune system. In Buckingham JC, Gillies GE, Cowell A (eds): Stress, Stress Hormone and the Immune System. Wiley, New York, 1993, pp. 199–223.
39. Yeager MP,Pioli PA,Wardwell K,Beach ML,Martel P,Lee HK, *et al* .*In Vivo* Exposure to high or low cortisol has biphasic effects on inflammatory response pathways of human monocytes. Anesth Analg. 2008; 107(5): 1726-34.