www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(8): 102-106

www.thepharmajournal.com
Received: 19-08-2015
Accepted: 21-09-2015

Nilotpal Mandal
Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India.

Goutam Dutta
Department of Physiology, University College of Science and Technology, University of Calcutta, 92 A.P.C. Road, Kolkata 700009, India.

Anandaraj Goswami
Department of Physiology, University College of Science and Technology, University of Calcutta, 92 A.P.C. Road, Kolkata 700009, India.

Tusharkanti Ghosh
Department of Physiology, University College of Science and Technology, University of Calcutta, 92 A.P.C. Road, Kolkata 700009, India.

Correspondence
Nilotpal Mandal
Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India.

Effects of lesion of Amygdaloid nuclei on blood immunocytes in male albino rats

Nilotpal Mandal, Goutam Dutta, Anandaraj Goswami, Tusharkanti Ghosh

Abstract
The regulatory role of nervous system on immune system has been emphasized by several investigators. In the present study, the role of basolateral nucleus (BLP), central nucleus (CeA) and medial nucleus (MeA) of amygdala on Total count (TC) of WBC, Differential Count (DC) of WBC and Arneth count of nutrophil were studied after two weeks of lesion of these nuclei of amygdala by electrolytic method in separate groups of rats. The results of these lesioned rats were compared with that of control and sham operated rats. Though the TC of WBC was not altered after lesion of any one of the three amygdaloid nuclei. The percentage of lymphocyte was significantly increased in BLP lesioned rat and decreased in CeA lesioned rats that of the control and sham operated rats. The percentage of neutrophil was significantly decreased in BLP lesioned rat compared to that of control and sham operated rats after two weeks of operation. In Arneth count the percentage of 3 lobed and 4 lobed neutrophils was significantly decreased while the percentage of 6 lobe and 7 lobed was significantly increased in BlP lesioned rats compared to that of control and sham operated rats after two weeks of operation. It appears from this study that BLP and CeA of amygdale exhibit a modulatory role on blood immunocytes and this immunomodulatory role of different nuclei of amygdale is not similar.

Keywords: Amygdala, Immune response, WBC, Arneth count.

Introduction
The amygdala is a part of the limbic system most specifically involved with emotional experience (Morris *et al*., 1996, Kandal *et al*., 2000) [1, 26]. Electrical stimulation of amygdala in humans produces feeling of fear. Conversely damage to amygdala in experimental animals produces tameness (Kandel *et al*., 2000) [26]. In humans lesions of amygdala occur as a part of the wide spread Urbach-Wiethe disease, a degenerative condition associated with calcium deposition in amygdala. This disease disrupts unconscious processing of cues of fear (Kandel *et al*., 2000) [26]. Amygdala is not only a centre of implicit conditioned memory; it has direct neural connection to the autonomic preganglionic neurons, hypothalamus and septum (Ader *et al*., 1996) [33]. It is receiving projection from vagus nerve (Ge *et al*., 2001) [16]. The cytokine expression may be induced in amygdaloid nuclear complex by immunization (Gao *et al*., 2000) [15] and it suggests that the neurons in this area are involved in possible immune regulation.

Basolateral amygdala (BLP) has a critical role on memory consolidation. Lesions of the basolateral complex block the acquisition and expression of fear conditioning (Canteras *et al*., 1992 [6], Ledoux *et al*., 1990., maren *et al*., 1999) [27, 29] and lesion of central nucleus of amygdala (CeA) inhibits the expression of fear conditional responses using visual or auditory system (Goosens *et al*., 2001) [18]. CeA is also involved in the modulation of attention, arousal & vigilance during conditioning and CeA has a significant role in generating both the mPVN (medial para ventricular neucleus) and CRF (Cortico tropin releasing factor) cell response, and ACTH release following systemic IL-1β administration (Xu *et al*., 1999) [49]. The role of BLP and CeA on behavior and immune system was studied after lesion of these nuclei of amygdale in rat (Grijalva *et al*. 1990) [50]. It was reported that NKCC was not significantly clanged in these lesioned animals through CeA lesioned rats were over active and BLP liseoned ones were hyperactive. (Grijalave *et al*. 1990) [50]. The amygdala has direct neuronal connections to autonomic preganglionic neuron, hypothalamus and septum (Ader *et al*., 1996) [33]. It is also connected to immunomodulatory areas such as hippocampus, striatum, mid brain, nucleus accumbens, and cerebral cortex (Mcdonald *et al*., 1998; Pitkanen *et al*., 2000 [28, 39]; Price *et al*. 1987, Mascagni *et al*. 1993) [30, 40]. Different neucli of amygdale such as basolateral, central and medial have different role on behaviour and are connected to different area of brain.

BPL is connected to hippocampus and polymodal association cortex, prefrontal cortex. CeA is connected to viscero sensory cortex, prefrontal cortex and MeA is connected olfactory bulb (Jolkkonen *et al*., 1998) [51].
Considering the neural connections of BLP, CeA and MeA of amygdala with autonomic neural regulating centers and/or immunomodulatory areas such as hypothalamus, septum, hippocampus, thalamus and midbrain it is pertinent to a investigate the changes of blood immunocyte such as lymphocytes, monocytes and neutrophils.

Methods

Animals

In this study 42 male albino rats (Charles-Foster strain) weighing 200–220 g 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. According to the Institutional Animal Ethical Committee all adequate measures were taken to minimize the pain and discomfort to the rats.

Design of Experiments

Experiment I: TC of Blood WBC, DC of Blood WBC and Arneth count of Nutrophil

Eighteen rats were equally divided into 3 groups: control, sham-operated and BLP lesioned rat. Total count (TC) of WBC, differential count (DC) of WBC and Arneth count of Nutrophils were measured in BLP lesioned and sham-operated groups 2 weeks after surgery and also in control group.

Experiment II: TC of Blood WBC, DC of Blood WBC and Arneth count of Nutrophil

Twelve rats were equally divided into 2 groups: sham-operated and CeA lesioned rats. Total count (TC) of WBC, differential count (DC) of WBC and Arneth count of Nutrophils were measured in CeA lesioned rats and sham-operated groups 2 weeks after surgery.

Experiment III: TC of Blood WBC, DC of Blood WBC and Arneth count of Nutrophil

Twelve rats were equally divided into 2 groups: sham-operated and MeA lesioned rats. Total count (TC) of WBC, differential count (DC) of WBC and Arneth count of Nutrophils were measured in MeA lesioned rats and sham-operated rats 2 weeks after surgery.

Blood Collection

The blood was collected (0.5 ml) from the heart of a deeply anesthetized rat (Na-thiopentone, 50 mg/kg body weight, i.p.) by a syringe containing 100 µl of Na-citrate (3.8%, Sigma) between 2: 30 and 3: 00 p.m. on the day of sacrifice (2 or 3 weeks after surgery). 1.5 ml of blood was also collected subsequently. The blood was mixed with 0.1 g ethylenediaminetetraacetic acid for the determination of TC, DC of WBC and Arneth count of Nutrophil.

TC, DC of WBC and Arneth count of Nutrophil

The TC of WBC was measured with the help of a Neubauer hemocytometer [WHO, 2000] [47]. The DC of WBC and Arneth count of Nutrophils were determined microscopically on the blood film stained with Leishman stain (Merck) [WHO, 2000] [47].

Confirmation of Lesion by Histology

The animals were sacrificed at the end of the experiment and were perfused intracardially with 0.9% saline followed by 10% formaldehyde solution. The brains were removed from the skulls and were kept in 10% formalin solution for fixation. After dehydration and clearing, paraffin blocks of those brains were prepared and 10- µ m-thick sections were cut by a microtome. The brain sections were stained by hematoxylin-eosin to identify the lesioned area (fig. 1, 2 and3).

Statistical Analysis

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

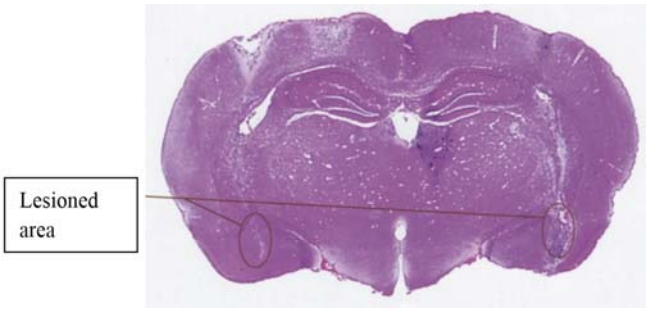

Fig 1: Hematoxylin-eosin-stained coronal histological section of rat brain showing the Amygdala (BLP) lesioned area.

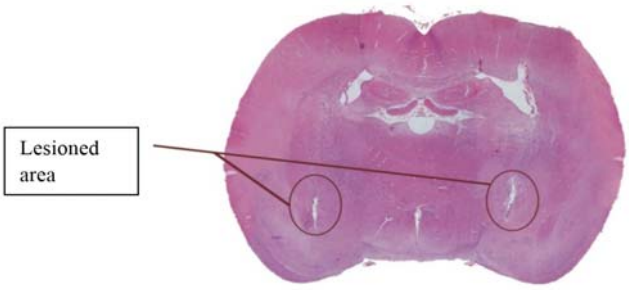

Fig 2: Hematoxylin-eosin-stained coronal histological section of rat brain showing the Amygdala (CeA) lesioned area.

Fig 3: Hematoxylin-eosin-stained coronal histological section of rat brain showing the Amygdala (MeA) lesioned area.

Results

Experiment I

The TC of WBC remained unaltered in the BLP lesioned rats (2 weeks after surgery) compared to that in the control and sham-operated groups. The percentage of Neutrophil was significantly decreased [$F(2, 15) = 7.75$, $P<0.001$] in the BLP lesioned rats (2 weeks after surgery) compared to that in the control and sham-operated groups [$F(2, 15) = 7.75$, $P<0.01$]

and lymphocyte was significantly increased [F(2, 15) = 7.75, P<0.001] in the BLP lesioned group (2 weeks after surgery) compared to that in the control and sham-operated groups [F(2, 15) = 7.75, P<0.01] (table 1). In the Arneth count the percentage of Neutrophil of 3 lobe and 4 lobe was decreased significantly in Amygdala(BLP) lesioned rats compared to that in the control [F (2, 17) =6.651,P<0.01 and [F (2, 17) = 14.186, P<0.001] and sham operated groups[F (2, 17) =6.651,P<0.001 and [F (2, 17) = 14.186, P<0.001] respectively where as 6 lobe and 7 lobe was increased significantly in Amygdala(BLP) lesioned rats compared to that in the control [F (2, 17) = 10.966,P<0.01 and F (2, 17) = 14.186, P<0.001] and sham operated groups[F (2, 17) = 10.966,P<0.001 and F (2, 17) = 14.186, P<0.001] (table 4)respectively.

Experiment II

The TC of WBC remained unaltered in the groups of CeA lesioned rats (2 weeks after surgery) compared to that in the control and sham-operated groups. The percentage of Lymphocyte was significantly decreased [(F (2, 17) = 7.163, P<0.01 and P<0.05] in the CeA lesioned group (2 weeks after surgery) compared to that in the control and sham-operated groups (table 2). In the Arneth count the percentage of Neutrophil remained unaltered in the groups of Amygdala (CeA) lesioned rats (2 weeks after surgery) compared to that in the control and sham-operated groups.

Experiment III

The TC and DC of WBC remained unaltered in the groups of MeA lesioned rats (2 weeks after surgery) compared to that in the control and sham-operated groups. In the Arneth count the percentage of Neutrophil also remained unaltered in the groups of CeA lesioned rats (2 weeks after surgery) compared to that in the control and sham-operated groups.

Table 1: T.C. and D.C of WBC in BLP lesioned group and sham operated group 14 days after surgery with the TC and DC in control rats.

Groups	T.C. of WBC/ µl	Neutrophils, %	Lymphocytes, %	Monocytes, %
Control	8,333±111.369	24.4±0.368	66.2±0.648	4.8±0.221
14 days after Sham operation	7,900±653.867	22.0±1.602	72.0±1.053	4.0±0.523
14 days after Amygdala(BLP) lesion	7,361±111.458	14.7±0.385*	78.7±0.425**	3.8±0.126

Values are expressed as mean ± SEM (n = 6 in each group). The percentage of Nutrophil was significantly decreased compared to control and sham-operated rats 2 weeks after surgery: * p < 0.01 and The percentage of Nutrophil was significantly increased compared to control and sham-operated rats 2 weeks after surgery**p < 0.001.

Table 2: T.C. and D.C of WBC in CeA lesioned group and sham operated group 14 days after surgery with the TC and DC in control rats.

Groups	T.C. of WBC/ µl	Neutrophils, %	Lymphocytes, %	Monocytes, %
Control	8,333±111.369	24.4±0.368	66.2±0.648	4.8±0.221
14 days after Sham operation	7,200±140.328	23.5±1.602	70.1±0.536	3.5±0.897
14 days after Amygdala(BLP) lesion	7,250±140.346	28.0±4.542	62.4±0.943*	5.3±3.72

Values are expressed as mean ± SEM (n = 6 in each group). The percentage of Lymphocyte was significantly decreased compared to control and sham-operated rats 2 weeks after surgery: *p < 0.05.

Table 3: T.C. and D.C of WBC in MeA lesioned group and sham operated group 14 days after surgery with the TC and DC in control rats.

Groups	T.C. of WBC/ µl	Neutrophils, %	Lymphocytes, %	Monocytes, %
Control	8,333±111.369	24.4±0.368	66.2±0.648	4.8±0.221
14 days after Sham operation	7,200±140.436	23.7±2.932	68.0±1.945	4.2±1.546
14 days after Amygdala(MeA) lesion	7,730±134.247	22.0±2.944	69.6±1.026	4.0±1.592

Table 4: Arneth count of neutrophil in BLP lesioned group and sham operated group 14 days after surgery with the Arneth count of neutrophil in control rats.

Groups	Neutrophils (%)			
	3 lobed	4 lobed	6 lobed	7 lobed
Control	19.8±2.62	33.6±3.01	15.8±1.34	4.6±0.82
14 days after Sham operation	14.0±2.33	34.0±2.42	14.0±2.34	4.0±0.54
14 days after Amygdala(BLP) lesion	10.2±0.55*	14.0±1.44**	23.1±2.93**	30.8±3.84***

Values are expressed as mean ± SEM (n = 6 in each group). The percentage of 3 lobe and 4 lobe of Nutrophil was significantly decreased compared to control and sham-operated rats 2 weeks after surgery: * p < 0.05 and **p<0.01 respectively. The percentage of 6 lobe and 7 lobe of Nutrophil was significantly increased compared to control and sham-operated rats 2 weeks after surgery***p < 0.001.

Table 5: Arneth count of neutrophil in amygdala (CeA) lesioned group and sham operated group 14 days after surgery with the Arneth count of neutrophil in control rats values following ± indicate S.E.

Groups	Neutrophils (%)			
	3 lobed	4 lobed	6 lobed	7 lobed
Control	19.8±2.62	33.6±3.01	15.8±1.34	4.6±0.82
14 days after Sham operation	19.0±0.52	34.2±2.87	18.0±2.53	3.8±0.75
14 days after Amygdala(CeA) lesion	23.3±3.96	32.7±2.82	11.0±0.23	3.3±0.85

Table 6: Arneth count of neutrophil in amygdala (MeA) lesioned group and sham operated group 14 days after surgery with the Arneth count of neutrophil in control rats values following ± indicate S.E.

Groups	Neutrophils (%)			
	3 lobed	4 lobed	6 lobed	7 lobed
Control	19.8±2.62	33.6±3.01	15.8±1.34	4.6±0.82
14 days after Sham operation	19.0±0.57	34.0±2.61	17.0±2.56	3.3±1.22
14 days after Amygdala(MeA) lesion	23.5±2.42	34.7±2.82	16.6±0.52	3.5±0.56

Discussion

In the present study the effect of lesion of amygdaloid nuclei (BLP, CeA, MeA) on white blood corpuscles (WBC), which control the specific and nonspecific immunity in our body have been studied. As limbic structure is involved for the control of emotional behavior and is connected with the immune regulations area of the hypothalamus. Hippocampus, striatum area of limbic structure is also involved in brain CRH release and action (Gray *et al.*1987; Whitnall *et al.,* 1993; Herman *et al.* 1994; Irwin *et al*., 1990) [52, 46, 21, 22].

In this study the total count of WBC in control, sham operated and amygdala lesioned animal has been observed and it is revealed that the total count of WBC showed no significant change 14 days after lesion of any one of the amygdaloid nuclei such as BLP, CeA, MeA lesion in rats. Jurkowski *et al*. in 2001 [24] also reported that the number of leukocyte was not changed after lesion of BLP but after electrolytic lesion of septum total no of leucocytes was decreased and After lesion of PVN decreased blood leukocyte number (Hefco *et al*., 1993)] [19]. The present study showed that lesion of CeA and MeA were not able to change TC of WBC like that of BLP. It is revealed from the DC of WBC of present study of that neutrophils was decreased and percentage of lymphocyte was increased in BLP lesioned grop, but the percent of lymphocytes was decreased in CeA lesioned group. However the DC was not changed in MeA lesion group, Though there is no report on the changes of DC of WBC after lesion of amygdala, it has been reported that electrolytic lesion of PVN of hypothalamus decreases the number of neutrophil (Hefco *et al*., 1993) [19]. Lesion of AH decrease the number of lymphocyte and lesion of lateral septul increases the number of lymphocyte (Jurkowski *et al*., 2001) [24]. Therefore results sµggested that the percentage of lymphocyte were oppositely affected by BLP and CeA and neutrophils were only affected by BLP. A differential regulation of imunocyte are evident from the present study.

The Arneth count of showed that three lobed and four lobed of nutrophils were decreased but six lobed and seven lobed nutrophils were increased in BLP lesioned rats. The Arneth count did not show any change in CeA and MeA groups. It is found that the percent of nutrophils were decreased in BLP lesion rats but Arneth count showed a shift to right in BLP lesion rats. Result probably indicate the neutrophil mediated innate immunity was potentiated.

The behavioral changes in BLP and CeA lesioned rats were not observed in this study. As reported by others activity of rats were differentially changed from these two nuclei (Grijlava *et al*. 1990) [50]. Behabioral changes are accompanied by alternation of HPA axis or autonomic neural activity. However neither of these two possibilities were explored in this study.

The exact mechanism of the complex immune changes in Amygdala (BLP and CeA) lesioned rats cannot be ascertained from the present study.

This study concludes that some nonspecific parameters of immunity are changed after lesion of amygdala. However three different nuclei of amygdala investigated in this study play different roles on the observed parameters of immune system. Lesion of basolateral amygdala appears to potentiate some immune process while lesion of central nuclei of amygdala is found to depress this process. Lesion of medial amygdala doesn't show any changes on the investigated parameters of immune systems.

Reference

1. Ader R, Madden K, Felten DL, Bellinger DL, Schiffer RB. Interactions between the brain and the immune system. psychoneuroroimmunology. IN Neuropsychiatry editors Fogel BS, Schiffer RB, Willams, Wilins Baltimors, 1996
2. 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.
3. Ge X, Yang Z, Duan L, Rao Z. Evidence for involvement of the neural pathway containing the peripheral vagus nerve, medullary visceral zone and central amygdaloid nucleus in neuro immunomodulation. Brain Res 2001; 28-914(1-2):149-58.
4. Goosens KA, Maren S. Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats, Learn. Mem 2001; 8(3):148-155.
5. 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.
6. Herman JP, Cullium WE, Watson SJ. Involvement of the bed nucleus of the stria terminalis in toxic regulation, Neuroendo. 1994; 6:433-442.
7. Irwin M, Haµger RL, Provencio M, Britton KT. Sympathetic nervous System mediates central corticotrophin-releasing factor induced suppression of natural killer cytotoxicity. J Pharmacol Exp ther. 1990; 255:101-107.
8. Jurkowzki M, Trojniar W, Barmau A, ciepielewski Z, Siemion D, Tokarski J. Pripheral blood natural killer cell cytotoxicity after damage to the limbic system in the rat. Brain Behav Immun 2001; 15(1):93-113.
9. Kandel ER, Schwartz JH, Jessell TM. Principles of neural science.Mc Graw – Hill, New Yark., 2000, 988-989.
10. 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.
11. Macdonald AJ. Cortical pathways to the mammalian amygdala. Prog Brain Res 1998; 55:257-332.
12. Maren S. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. Trends Neurosci 1999; 22:561-567.
13. Mascagni F, Macdonald AJ, coleman JR. Corticoamygdaloid and corticocortical projections of the rat temporal cortex: a Phaseolus vulgaris leucoagglutinin study. Neuroscience 1993; 57:697-715.

14. 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.
15. Pitkanen, A. Connectivity of the rat amygdaloid complex. In: The Amygdala: A Functional Analysis, edited by Aggleton JP. Oxford, UK: Oxford Univ. Press, 2000, 31-11
16. Price JL, Russchen FT, Amaral DG. The Limbic Region. II: The Amygdaloid Complex. New York: Elsevier Science, 1987.
17. Whitnall M. Regulation of hypothalamic corticotrophin. Prog Neurobiology 1993; 40(11):573-629.
18. WHO Manual of basic techniques for a health laboratory. Academic publishers, Calcutta, 2000, 353-404.
19. Xu Y, Day, TA, Bullar KM. The central amygdala modulates hypothalamic –Pituitary- adrenal axis. Neurosci 1999; 94:175-183.
20. 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. Physiol. Behav. 1990; 48, 495-500.
21. Jolkkonen E, Pitkanen A. Intrinsic connections of the rat amygdaloid complex: projections originating in the central nucleus. J Comp Neurol. 1998; 395:53–72.
22. Gray TS, Magnuson DJ. Neuropeptide neuronal efferents from the bed nucleus. J Comp Neurolo. 1987; 262:365-374