Original Research Article

Stress effect on neurons of cingulate gyrus in postweaned albino mice - A histological study

Hemalatha Bangera[1], Prakash Babu B[2*], Antony Sylvan D'Souza[3], Manjula SD[4], Mohandas Rao K[5], Sushma RK[6]

[1]Junior Research Fellow, Department of Anatomy, Kasturba Medical College, Manipal University, Manipal, Karnataka, India
[2]Associate Professor, Department of Anatomy, Kasturba Medical College, Manipal University, Manipal, Karnataka, India
[3]Associate Dean and Professor, Department of Anatomy, Kasturba Medical College, Manipal University, Manipal, Karnataka, India
[4]Associate Professor, Department of Physiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India
[5]Professor and Head, Department of Anatomy, MM Medical College, Manipal University, Manipal, Karnataka, India
[6]Lecturer, Department of Anatomy, Kasturba Medical College, Manipal University, Manipal, Karnataka, India
[*]Corresponding author email: **billakantiprakashbabu@yahoo.com**

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Abstract

Stress is a common experience of daily life and all organisms have evolved mechanisms and strategies to deal with crucial alterations in their internal and external environment. Events early in post-weaning life influence brain development and subsequent adult behaviour. This study was conducted to observe neurodegeneration in normal and stressed condition in Postweaning age group of mice. Experiments were conducted to investigate the effect of restraint stress and foot shock stress applied for 5 days (short duration) and 21 days (long duration). BALB/ C strain of Albino mice were used. In restraint stress, animals were restrained for 6 hours daily in a restraining device. In foot shock group, animals were given intermittent electric foot shock for 3 hours daily in an electric foot shock apparatus. Animal were sacrificed at the end of stress exposure period along with their age matched control mice and the brains were processed for histological examination both in control and experimental groups. Numbers of viable neurons in the cingulate gyrus regions were counted. The

data were analysed between the groups. Results of the study revealed neurodegenerative changes in the stressed group compared to control in both the experimental groups. Severe neurodegenerative changes were observed on prolonged exposure to stress.

Key words

Albino mice, Restraint stress, Foot shock stress, Cingulate gyrus, Neurodegeneration.

Introduction

Stress is a highly individualized response of an organism to external or internal challenges which is possible or impossible to be controlled based on an individual's ability. Any stimulus that displaces the state of normal physiological function can cause stress [1]. Events early in post-weaning life influence brain development. Exposure to stress during the juvenile period can exert long-term effects on the brain and behaviour [2]. Any stimulus which displaces the state of normal physiological function can cause stress and has its effect in various forms [3]. Increased activity in the subgenual region of the anterior cingulate cortex which has been consistently linked with depression is related to heightened sensitivity to peer rejection among adolescents [4]. Acute and chronic stresses are characterized by the physiological changes that occur in response to novel or threatening stimuli. The neuroendocrine damages in response to acute and chronic stresses are mediated by both the sympathetic nerve system and the hypothalamus-pituitary-adrenal (HPA) axis [5]. Most of these studies have concentrated on the adult brain. However stress exposure in the postweaning period is seldom discussed. The present study was therefore an attempt to reveal the same.

Material and methods

Animals

In the present study, postweaning age group (21 days old pups) of albino mice of BALB/C strain of both sexes was used. Control and experimental groups consisting of six mice in each group were formed randomly. Ethical clearance was taken from institutional animal ethical clearance committee, Manipal University (IAEC/KMC/100/2012). The mice were maintained in institutional animal house, Manipal University.

Experimental design

The animals were divided into three subgroups.
(a) Control (C)
(b) Restraint stress (RS)
(c) Foot shock stress (FSS)

Both control and stress groups consisted of two sub groups according to duration of stress: Short duration stress (5 days stress group) and Long duration stress (21 days stress group).

In the control group, mice remained undisturbed in their home cage. In the restraint stress group (RS), the mice were stressed for 6 hours per day from postnatal day 22 to postnatal day 26 (5 days stress) and from postnatal day 22 to day 42 (21day stress) in a restraining device, which consisted of a wooden platform to which a wire-mesh was attached for 5 and 21days. In the foot shock stress group (FSS), mice were given intermittent electric foot shock for three hours per day from postnatal day 22 to postnatal day 26 (5days stress) and from postnatal day 22 to day 42 (21day stress) for 5 and 21 days.

Tissue procurement and processing

At the end of stress exposure period these mice were sacrificed along with their age matched control mice for histological studies. Each mouse was anesthetized with a high dose of ether and fixation was performed by trans-cardiac perfusion with 0.9% saline followed by 10% formalin. The brain was removed and kept in 10% formalin for 2 days post fixation. Paraffin blocks were made and coronal sections of 5μm thickness were cut using a rotary microtome. The sections were labelled and mounted on to air

dried gelatinised slides and were stained with cresyl violet [6].

Light microscopic examination

The stained slides were examined under 10X and 40X magnifications using a light microscope. The cingulate gyrus was identified with the help of Paxinos and Watson's atlas. Proper stained slides without artefacts in the regions of interest were considered for counting the neurons.

Cell counting

Ten sections from each mouse were selected for counting. Number of viable neurons in the Cingulate gyrus was counted with 40X magnification across 250µ length with the aid of ocular micro meter. All the slides were coded before the counting to avoid the manual bias. The results were expressed as number of viable cells per unit length of the field (Number of cells/250µ).

Statistical analysis

The data were analysed with one way Anova followed by Dunnets`s post-test to compare the experimental groups with control and Bonferroni's multiple comparison test to compare between all the groups using Graph Pad Prism, version 5 (Graph Pad Prism Software inc., USA). The results were expressed as Mean ± SD, p value less than 0.05 was considered statistically significant.

Results

Short duration stress

Results of the present experiment showed significant decrease in number of viable neurons in the cingulate gyrus in both restraint and foot shock stressed mice in comparison to their age matched control (p value < 0.0001). Further the numbers of viable neurons were significantly low in foot shock stressed mice compared to restraint stress group (p value < 0.0001) (**Figure – 1, 2**).

Long duration stress

Significant decrease in the number of viable neurons of the cingulate gyrus was observed in both restraint and foot shock stressed mice in comparison to their age matched control (p value < 0.0001). The severity of neurodegeneration in both restraint and foot shock stressed groups was similar and not statistically significant (**Figure – 3, 4**).

Neurodegeneration of the cingulate gyrus was seen in both the stress groups exposed to shorter and longer durations. Further the numbers of viable neurons were less in stress groups of longer duration compared to shorter duration. This indicated that the severity of neurodegeneration increased with the duration of stress.

Figure - 1: Cresyl violet stained histomicrographic picture taken under 40X magnification showing coronal sections of cingulate gyrus region of 5 days restraint stressed and foot shock stressed post weaned mice compared to the age matched control. (C-control, RS-restraint stress, FSS-foot shock stress)

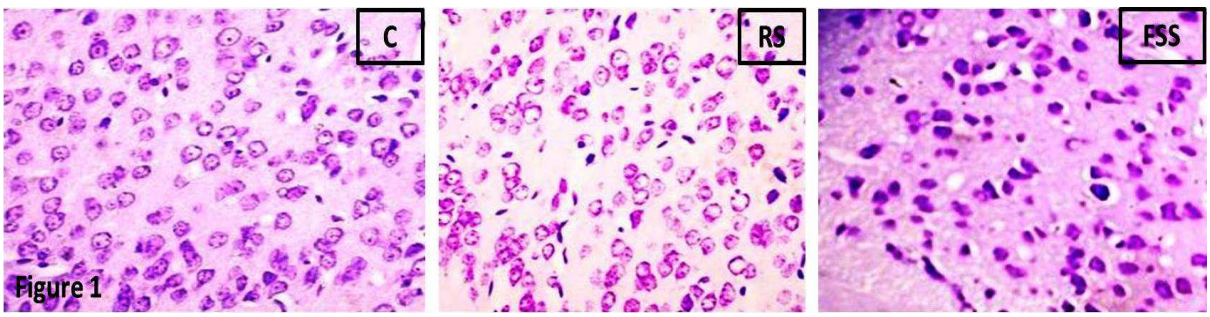

Figure - 2: Graphical representation of the number of viable neurons across 250 micron length in cingulate gyrus region of 5 days restraint stressed and foot shock stressed post weaned mice in comparison to their age matched control.
Each data represents Mean±SD. *p value<0.0001 compared to control, **p value<0.0001 compared to RS. CG-cingulate gyrus-control, RS-restraint stress, FSS-foot shock stress

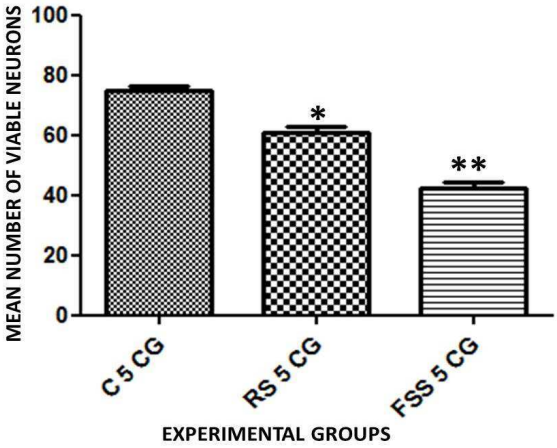

Figure 2

Figure - 3: Cresyl violet stained histomicrographic picture taken under 40x magnification showing coronal sections of cingulate gyrus region of 21 days restraint stressed and foot shock stressed post weaned mice compared to the age matched control. (C-control, RS-restraint stress, FSS-foot shock stress)

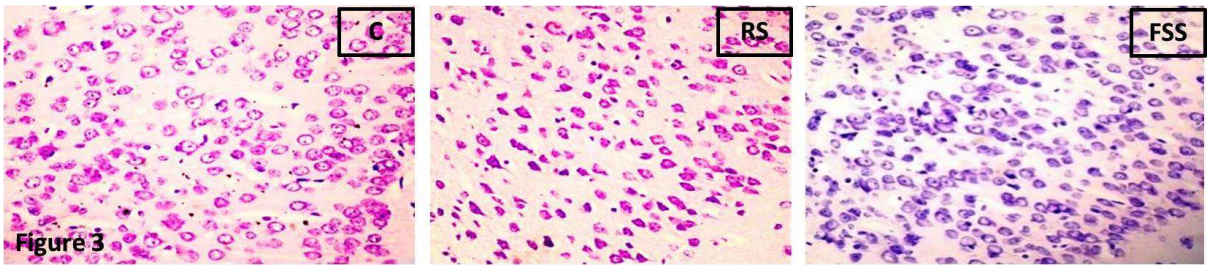

Figure 3

Figure - 4: Graphical representation of the number of viable neurons across 250 micron length in cingulate gyrus region of 21days restraint stressed and foot shock stressed post weaned mice in comparison to their age matched control. Each data represents Mean±SD. (CG-cingulate gyrus-control, RS-restraint stress, FSS-foot shock stress)

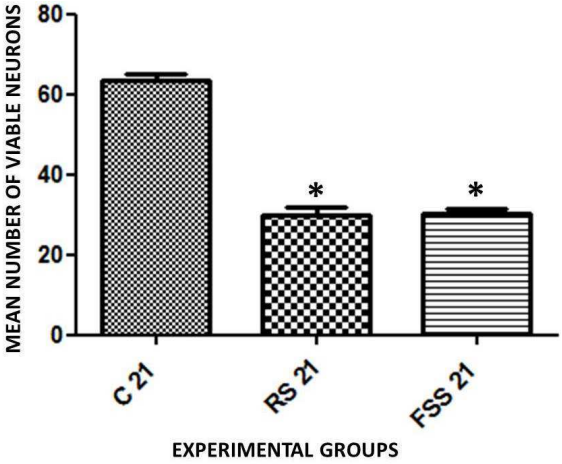

Figure 4

Discussion

Stress early in postnatal life may result in long-term memory deficits and selective loss of hippocampal neurons. The mechanisms involved are poorly understood, but they may involve molecules and processes in the immature limbic system that are activated by stressful challenges [7].

The present study was done to see the effects of duration of restraint stress and foot shock stress on cingulate gyrus neuronal morphology in post weaning group of albino mice. Results revealed extensive neurodegeneration in both restraint and foot shock stress of short and longer duration in comparison to their age matched control mice. Stress exposed brain showed histopathological changes suggesting necrosis/ apoptosis. The neurodegeneration observed in the present study was similar to histopathological changes observed in this experiment.

Short duration stress exposed animal brain showed less degeneration in restraint stress than foot shock stress. In long duration stress, there was significant loss of neurons in both restraint and foot shock.

Previously authors have studied the effect of restraint stress and chemical caused neuronal cell death on the cingulate cortex and showed neuropathological changes which was highly conspicuous with decreased number of surviving neurons (27–40% reduction**)**and the presence of dying neurons (4–10% of total neurons) [8].

Restraint-induced structural changes were also studied by the authors where the adult rat was exposed to 6 hours of restraint stress daily for 21 days causing marked morphological alterations in the medial prefrontal cortex [9].

Another study revealed chronic stress impaired hippocampal neurogenesis in mice in terms of cell proliferation; apoptosis; the number and maturation of young neurons; and both the volume and neuronal density in the granular zone [10].

In a study on the prefrontal cortex, the total number of the neurons and glial cells was significantly reduced (11% and 5%, respectively) in stress group in comparison to the non-stressed rats [11].

Conclusion

In conclusion we observed neurodegenerative change in the cingulate gyrus of stress exposed animals of postweaning age group. The findings of the present experiment were consistent with the findings of the previous researchers. This study will have an implication in understanding the patho-psychology of stress related disorders and the harmful impact it has on early brain development.

References

1. Fisher H, Morgan C, Dazzan P, Craig TK, Morgan K, Hutchinson G, et al. Gender differences in the association between childhood abuse and psychosis. Br J Psychiatry, 2009; 194(4): 319–325.
2. Peleg-Raibstein D, Feldon J. Differential effects of post-weaning juvenile stress on the behaviour of C57BL/6 mice in adolescence and adulthood. Psychopharmacology (Berl)., 2011; 214(1): 339-51.
3. Prakash billakanti babu, Ramachandra Bhat K, Muddanna S Rao, et al. Longitudinal study on the effect of restraint stress during preweaning period on postnatal growth of body and constituent parts in albino mice. Int. J. Pharm. Med. & Bio. Sc., 2012, 1(2): 2278 – 5221.
4. Carrie Mastena, Naomi I. Eisenbergera, Larissa A. Borofskyb, Kristin Mcnealyc, Jennifer H. Pfeiferd, Mirella daprettoa, et al. Subgenual anterior cingulate responses to peer rejection: A marker of adolescents' risk for depression.

Development and Psychopathology, 2011; 23(1): 283-92.

5. Imperato, Angelucci, Casolini, Zocchi, Puglisi-Allegra, et al. Repeated stressful experiences differently affect limbic dopamine release during and following stress. Brain Res., 1992; 17: 194–199.
6. Madhyastha S, Bairy KL, Nalini K, Somayaji SN. Hippocampal brain amines in methotrexate-induced learning and memory deficit. Canadian J Physiol Pharmacol., 2002; 80(11): 1076-84.
7. Kristen L. Brunson, Mariam Eghbal-Ahmadi, Roland Bender, Yuncai Chen, Tallie Z. Baram, et al. Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. The National Academy of Sciences, 2001; 98(15): 8856-8861.
8. Ali abdel-rahman, Ashok K. Shetty, Mohamed B Abou-Donia, et al. Disruption of the Blood–Brain Barrier and Neuronal Cell Death in Cingulate Cortex, Dentate Gyrus, Thalamus, and Hypothalamus in a Rat Model of Gulf-War Syndrome. Neurobiology of Disease, 2002; 10: 306–326.
9. Meng-Yang Zhu, Wei-Ping Wang, Zheng-Wei Cai, Soundar Regunathan, Gregory Ordway, et al. Exogenous agmatine has neuroprotective effect against restraint-induced structural changes in the rat brain. Eur J Neurosci, 2009, 27(6): 1320-1332.
10. Estela Castilla-Ortega, Carolina Hoyo-Becerra, Carmen Pedraza, Jerold Chun, Fernando Rodrı´guez De Fonseca, Guillermo Estivill-Torru S, Luis J. Santı´n, et al. Aggravation of Chronic Stress Effects on Hippocampal Neurogenesis and Spatial Memory in LPA1 Receptor Knockout Mice. PLOS ONE., 2011; 6(9): e25522.
11. Ali Noorafshan, Mohammad-Amin Abdollahifar, Reza Asadi-Golshan, Ali Rashidian-Rashidabadi, Saied Karbalay-Doust, et al. Curcumin and sertraline prevent the reduction of the number of neurons and glial cells and the volume of rats' medial prefrontal cortex induced by stress. Acta Neurobiol Exp (Wars), 2014; 74(1): 44-53.