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THE EFFECT OF SHORT AND LONG DURATION OF RESTRAINT STRESS ON NEURODEGENERATION OF CINGULATE CORTEX AND SUBSTANTIA NIGRA IN ADULT ALBINO MICE- HISTOMORPHOLOGICAL STUDY

Hemalatha Bangera, Billakanti Prakash Babu & Antony Sylvan D'Souza

Department of Anatomy, Kasturba Medical College
Manipal University, Manipal-576104, Karnataka,
India

Manjula S D

Department of Physiology, Kasturba Medical
College Manipal University, Manipal-576104, Karnataka,
India

Mohandas Rao K

Department of Anatomy, Melaka Manipal Medical
College Manipal University, Manipal-576104, Karnataka,
India

ABSTRACT

Any stimulus which displaces the state of normal physiological function can cause stress and it has been shown to produce deleterious effect on the brain just as it does in other body organs. This study was conducted to observe the effect of short and long duration of restraint stress on neurons of cingulate gyrus and substantia nigra. Experiments were conducted to observe the effect of restraint stress applied at different duration i.e. short duration of 5 days and longer durations of 21 and 60 days. BALB/C strain of adult mice were restrained for 6 hours daily for 5/21/60 day in separate group in wire mesh restrainer which consisted of a wooden platform to which a wire mesh was attached. Age matched normal mice served as control group. Numbers of surviving (healthy) neuronal cell bodies were counted in both control and experimental groups. Recorded parameters were subjected to statistical analysis between the groups. The results of the study showed neurodegenerative changes in the stressed group than control and as the stress duration increased severe neurodegenerative changes were observed. Significant neurodegenerative changes are seen in 60 day stressed cingulate gyrus sections compared to others.

General Terms

Restraint Stress, Neurodegeneration, Cingulate Cortex, Substantia nigra, Albino Mice, Histomorphology.

Keywords

Restraint Stress, Neurodegeneration, Cingulate Cortex, Substantia nigra, Albino Mice, Histomorphology.



1. INTRODUCTION

Stress is a highly individualized response of an organism to external or internal challenges which individual cannot control or can control with difficulty. Any stimulus that displaces the state of normal physiological function can cause 'stress. (Fisher et al., 2009).

Increased activity in the subgenual region of the anterior cingulate cortex (cingulate gyrus), which has been consistently linked with depression is related to heightened sensitivity to peer rejection among adolescents. (Carrie et al., 2011).

Substantia nigra neurons are susceptible to stress induced damage. It is the duration of stress which is important in causing the neuronal damage than the nature of stress (Babu et al., 2012).

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, (Imperato et al., 1992).

Chronic stress is known to impair memory and to reduce neurogenesis. However, the effects of acute stress are less clear-cut: early studies suggested that it suppressed the generation of new neurons, whereas several recent studies have observed no effect. Other work has shown that acute stress increases the expression of growth factors—substances that stimulate cellular growth and proliferation—which would seem to suggest that stress could enhance neurogenesis (Elizabeth D Kirby et al., 2012).

2. MATERIALS AND METHODS

Animals

In the present study adult (270 days) albino mice of BALB/C strain of both sexes were used. Control and experimental group consisting of twelve albino mice in each group were formed randomly. Ethical clearance was taken from an institutional animal ethical clearance committee, Manipal University (no. IAEC/KMC/100/2012). The mice were maintained in institutional animal house Manipal University.

Stress Regimen

The adult mice were divided into two subgroups

(a) Control (C)

(b) Restraint stress (RS)

Control Group-Mice remained undisturbed in their home cage

Restraint Stress Group (RS)-Mice were stressed in restrainer No-5 having dimensions –6.5 cm (L) x 4.0 cm (B) x 3.8 cm (H), for 6 hours per day for 5 or 21 or 60 days

Both control and restraint stress group consist of three sub groups according to duration of stress as

5 days stress group

21 days stress group

60 days stress group



All the mice in the restraint stress group remained in their home cage except when they were subjected to stress. Control mice remained undisturbed. The food and water were withdrawn from experimental group during stress presentation. After stress presentation mice were returned to their respective cages.

Tissue 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 and 10% formalin. The brain was removed and kept in 10% formalin for 2 days post fixation. Paraffin blocks were made and coronal sections of 5 μ m thickness were cut in the using a rotary microtome. The sections were labelled and mounted onto air dried gelatinised slides. Slides are stained with cresyl violet (Madhyastha et al., 2002)

Cresyl Violet Staining Procedure

We have followed the method described by the Madhyastha et al., 2002

Light Microscopic Examination

The stained slides were examined under 10X and 40X with light microscope; the cingulate gyrus and substantia nigra regions were identified with help of Paxinos and Watson. Proper stained slides without artefacts in the regions of interest were considered for counting the neurons.

Cell Counting

Ten sections from the each mouse for the each area were selected for counting. Number of viable neurons in the Cingulate gyrus and substantia nigra regions were counted with 40X magnification across 250 μ length with the aid of ocular micrometer. 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/250 μ).

3. STATISTICAL ANALYSIS

From these Cresyl violet stained sections, healthy neuronal cell bodies were counted in 10 sections per animal in both cingulate gyrus and substantia nigra regions. The average was noted. The data were analysed with Independent sample T test using SPSS v16. The results were expressed as Mean \pm SD, p value less than 0.05 was considered statistically significant.

4. RESULTS

Cingulate Cortex Region

Results of present experiment showed significant decrease in number of viable neurons in 5, 21, and 60 days restraint stressed mice cingulate gyrus section in comparison to their age matched control mice. The numbers of viable neurons were less in stress of longer duration than short duration (severity of neurodegeneration increased with the duration of stress) There was significant loss of neurons ($p < 0.005$) in cingulate gyrus section of 60 day stressed mice compare to other groups.



Substantia nigra Region

Results of present experiment showed restraint stress caused There was significant decrease in number of viable neurons in 5, 21, 60 days restraint stressed mice substantia nigra section in comparison to their age matched control mice. The numbers of viable neurons were less in stress of longer duration than short duration (severity of neurodegeneration increased with the duration of stress).

5. DISCUSSION

The present study was done to see the effects of three different duration of restraint stress on cingulate gyrus and substantia nigra neuronal morphology in adult albino mice. Results revealed extensive neurodegeneration in the cingulate gyrus than substantia nigra and the chronic stress of 21 and 60 days than acute stress of 5 days.

Short duration stress (5 day restraint) received animal brain showed less degeneration while 21 and 60 days restraint 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.

A study on Restraint-induced structural changes in the rat brain in which 6 h of restraint stress daily for 21 days caused marked morphological alterations in the medial prefrontal cortex (Meng-Yang Zhu1 et al., 2009)

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 (Estela Castilla-Ortega et al 2011)

A study on prefrontal cortex the total number of the neurons and glial cells was significantly reduced (11% and 5%, respectively) in stress (+distilled water or olive oil) group in comparison to the non-stressed rats (Ali Noorafshan et al., 2014)

The number of neurons in the substantia nigra pars compacta region was significantly decreased in chronic restraint stress than acute stress, which is similar to results of our study (Babu et al., 2002).

Another study observed chronic stress sensitizes midbrain microglia, which enhances microglia activation and exacerbates death of nigral dopaminergic neurons to further inflammatory stimulus. (Rocio et al., 2014)

Acute stress increases the expression of growth factors—substances that stimulate cellular growth and proliferation—which would seem to suggest that stress could enhance neurogenesis (Elizabeth D Kirby et al., 2013)

However from results of our study the effects of acute stress are less clear-cut. Early studies suggested that it suppressed the generation of new neurons

6. CONCLUSION

In conclusion we observed neurodegenerative change in cingulate gyrus and substantia nigra regions of stress exposed animals in comparison to their age matched control animals of adult age group. These changes were less severe in short duration stress received animals. The findings of the present experiment were consistent with the findings of the previous researchers. This study will have an implication in understanding pathopsychology of stress related disorders.



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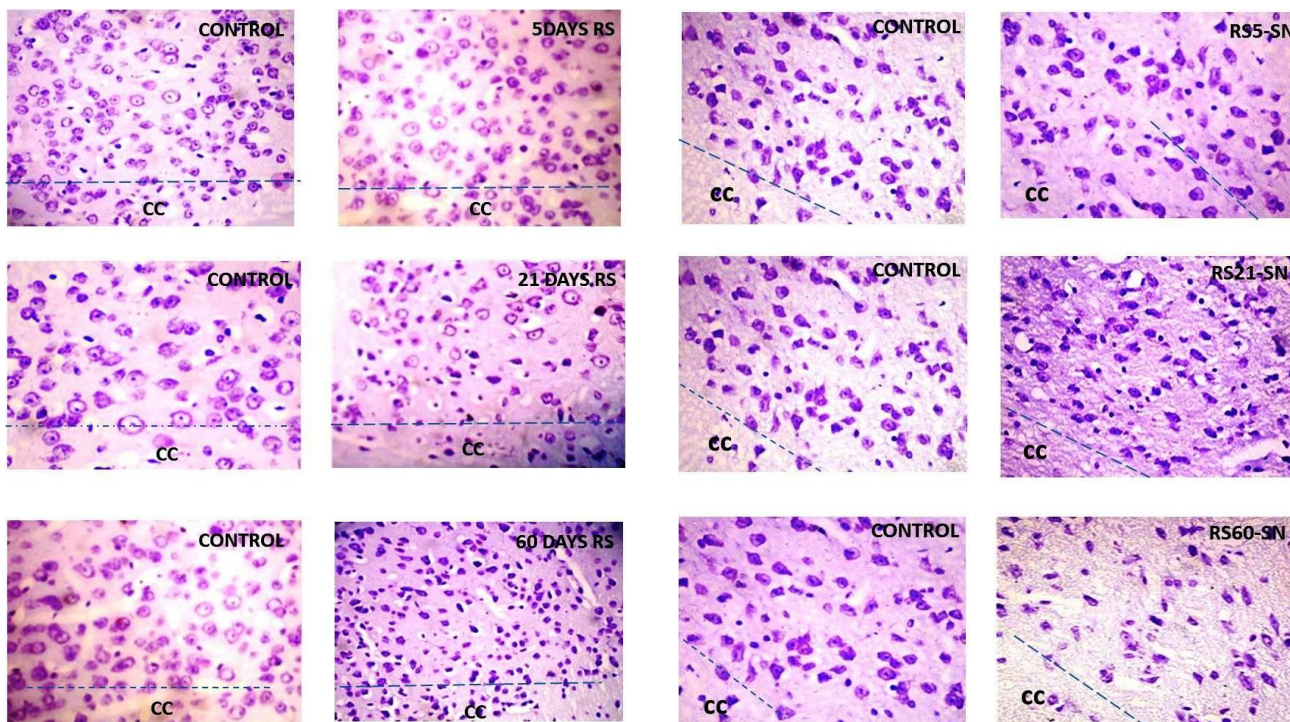


Fig 1.Histomicrographic picture taken under 40x showing cresyl violet stained coronal sections of cingulate gyrus region of 5, 21 and 60 days restraint stressed mice compared to the age matched control mice (cc-corpora collosum)

Fig 3.Histomicrographic picture taken at 40x showing cresyl violet stained coronal sections of substantia nigra region of 5,21 and 60 days restraint stressed mice compared to the age matched control mice.(cc-crus cerebri)

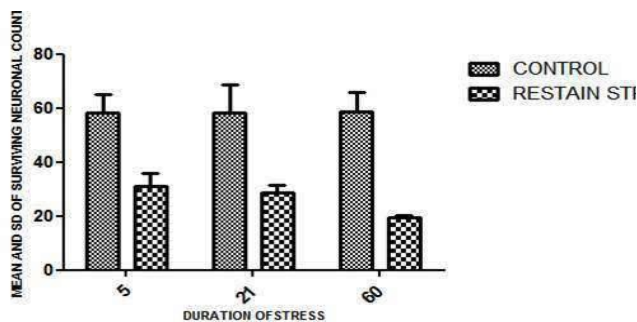


Fig 2 Bar graph shows the number of viable neurons across 250 micron length in cingulate gyrus region of 5,21,60 day stressed group in comparison to their age matched control. Each data represents Mean±SD.

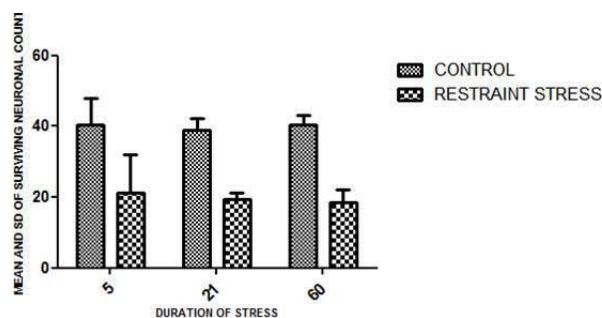


Fig 4 Bar graph shows the number of viable neurons across 250 micron length in substantia nigra region of 5,21,60 day stressed group in comparison to their age matched control. Each data represents Mean±SD