ACUTE AND CHRONIC IMMOBILIZATION STRESS; EFFECTS ON CEREBELLAR CORTEX OF YOUNG MALE SPRAGUE DAWLEY RATS

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ABSTRACT... Objective: The purpose of this article is to compare the morphological changes induced by acute immobilization with those produced by chronic immobilization, in vermal cerebellar cortex of young male sprague dawley rats. **Study design:** Experimental study. **Setting:** Department of Anatomy CPSP Regional Centre, Islamabad. **Period:** 2005 to 2007. **Material and method:** A total of 120 young male Sprague Dawley rats were taken and divided equally in two groups for acute and chronic immobilization stress. Both these groups were divided further into two subgroups (n=30 in each) control acute, experimental acute, control chronic and experimental chronic. 'Experimental acute' group Ib was kept in restrainer for 24 hours continuously while 'experimental chronic' group IIb was kept in restrainer for 2 hours daily for fourteen days, one rat per restrainer separately. Hematoxylin and Eosin stained sections were studied under light microscope to record the data. Results of experimental acute group were compared with those of experimental chronic'. Data was analyzed by SSPS version 10. **Results:** Insignificant increase in thickness of vermal cerebellar cortex and molecular layer was found in both acute and chronically stressed rats; but comparatively more in group Ib, however minimal non significant change in granular layer was seen in both experimental groups. **Conclusions:** Immobilization stress can cause the neuronal injury. Acute stress tends to produce more severe effects on brain cortical tissue as compared with chronic stress. The reason for occurrence of less severe effects under chronic stress is probably due to role of the phenomena of adaptation.

Key words: Acute, Chronic, Immobilization stress, Purkinje cells and vermal cerebellar cortex.

.INTRODUCTION

Immobilization limits the movements of the body, which can affect the body systems and can produce various pathological states. A number of diseases are related to immobilization under conditions of chronic stress that can cause the degenerative diseases such as, aging, and many other brain dysfunctions[1]. Immobilization affects multiple systems within the CNS. Learning and memory have been reported to be affected under the effect of chronic stress in rats differentially[2]. The normal development and preservation of human life and species are dependent on a normally functioning stress system. It has been noticed that chronic stress or chronic use of glucocorticoids induces dendritic remodeling in prefrontal pyramidal neurons[3] which can cause different health problems, such as psychiatric, endocrinal and metabolic disorders.

In a comparative study, people have found increase in corticosterone level in acute stress while decrease in chronic stress[4]. Stress produces oxidants especially in the brain, which cause damage to the cellular molecules such as, lipids and protein in the brain of the rat[5]. Immobilization stress induces oxidative damage in rats and antioxidant administration effectively inhibits this damage[6]. Immobilization is a well-known model of limiting movements in laboratory animals. Studying the effects of immobilization stress in rodents, especially in rats are more familiar[7]. Sapolsky and co-workers[8,9,10,11] have studied adverse effects on different brain regions, like hippocampus amygdala and cerebellum. Similarly McEwen et al[12] has discussed the effects of stress on brain cells which are affected by glucocorticoids, released during stress. Significant dendritic remodeling of pyramidal neurons has been also noted[13] under the effect of chronic immobilization stress in the rat hippocampus and amygdala[14].

Although, immobilization induced changes in different parts of the rat cerebral cortex such as hippocampus, amygdala, prefrontal cortex and lobes of rat cerebellum have been reported earlier but no reports are available on comparative study of effects of acute and chronic immobilization stress on vermal cerebellar cortex of young male rats. The present project was designed to observe and compare the effects of acute and chronic immobilization stress on histological changes of the thicknesses of various layers of of vermal cerebellar cortex of young male sprague dawley rats.

MATERIAL AND METHODS

Study design of this project was experimental. It was carried out at Anatomy Department of CPSP Regional Center Islamabad from 2005 to 2007. Young male healthy Sprague Dawley rats of 12 weeks age, weighing about 200-230 g, were purchased from National Institute of Health Islamabad. They were kept at CPSP Regional Centre Islamabad under controlled conditions. These animals were housed singly in their home cages for adaptation to the new environment for a week. They were fed on the standard diet (available on commercial basis from National Institute of Health Islamabad) and tap water ad libitum. For experimental procedure, locally made immobilization device was used as a restrainer. For study purpose, a total of 120 young male animals were equally divided into two groups i.e. for acute. (Group I) and chronic (Group II) immobilization stress.

Group I: In this group there were 60 animals, which were divided in two subgroups. Control acute subgroup (Ia) = 30 animals. Experimental acute subgroup (Ib) = 30 animals.

Group II: In this group there were 60 animals, which were divided in two subgroups. Control chronic subgroup (IIa) = 30 animals. Experimental chronic subgroup (IIb) = 30 animals.

On the day of experiment for AIS, the animals of control (Ia) and experimental (Ib) subgroups were removed from their home cages and weighed. Animals of control (Ia) subgroup were placed into their labeled cages, one animal per cage, for the period of 24 hours in continuation. They were free to move in their cages, but the animals of experimental (Ib) subgroup were immobilized, separately by placing them into their labeled restrainers, one animal per restrainer. The period of immobilization was for 24 hours continuously. Animals of both subgroups (Ia) and (Ib) were supplied only water and their food was restricted during experiment. After the completion of experimental procedure, animals of subgroups (Ia) and (Ib) were removed from their cages and restrainers respectively, and all the rats of both subgroups were weighed, anaesthetized and operated on the same day, one by one on their turn, under ether anesthesia. At the end of the procedure, the whole brain with cerebellum was removed and stored into the labeled bottle, filled with 10% formalin as a fixative.

Similarly on the day of experiment for CIS, the animals of control (IIa) and experimental (IIb) subgroups were removed from their home cages and weighed. Animals of control (IIa) subgroup were placed into their labeled cages, one animal per cage, for the period of 2 hours. These rats were free to move in their cages, but the animals of experimental (IIb) subgroup were immobilized separately by placing them into their labeled restrainers, one animal per restrainer. The period of immobilization was 2 hours, for 14 days. Animals of both subgroups (IIa) and (IIb) were supplied only water and their food was restricted during the period of experiment. After the completion of experimental procedure, the animals of subgroup (IIa) were removed from their cages and put back into their home cages while the animals of experimental subgroup (IIb) were removed from their restrainers and put back into their home cages as before where feed was allowed. On the other day, same procedure was repeated with the animals of control and stressed subgroups. Soon after the completion of the experimental protocol for fourteen days, all the rats of control and experimental subgroups of CIS were weighed, anaesthetized and the whole brain with cerebellum was removed, stored into the labeled bottle, filled with 10% formalin.

The vermis and the cerebellar hemispheres were identified. A piece of vermal tissue was processed to get the sections in parasagittal plane, for measurements of thicknesses of various layers of the cerebellar cortex. Paraffin embedding was done for block formation 6. Five µm thick sections were cut, stained with Hematoxylin and Eosin for examination under light microscope. P-value was found by applying SPSS version 10. Student's t test was applied to detect any significant differences in the means of the parameters of the quantitative data.

RESULTS

The animals of immobilized group I were depressed and showed hypoactivity more as compared to their immobilized group II, when removed from their restrainer after continuous immobilization stress of 24 hours and 2 hours respectively. Rats of stressed group I were not

showing any interest in food intake as compared to group II, which showed little change for a few days but after that their habit for food intake was normal. The plastic bottles of the experimental animals of group I were filled more with urine and fecal matter as compared to experimental animals of group II. Apparently there was no difference in the gross anatomy of the cerebellum of both control and immobilized animals of both acute and chronic groups.

Increase in thickness of vermal cerebellar cortex and molecular layer was found in stressed rats of both acute and chronic immobilization stress but the difference between the thickness of vermal cerebellar cortex in experimental acute (414.51 ± 9.37 µm) and control acute (400.07 ± 9.34 µm) subgroup was more as compared with the difference between the experimental chronic (426.92 ± 9.38 µm) and control chronic (417.77 ± 9.34 µm) subgroups. Similarly difference between the thickness of molecular layer in experimental acute (195.00 ± 5.69 µm) and control acute (183.21±5.28 µm) subgroup was more than the difference between the experimental chronic (201.22 ± 5.17 µm) and control chronic (194.67 ± 4.04 µm) subgroups. Differences between the experimental acute and experimental chronic subgroups were statistically insignificant ($p > 0.05$), but these changes were more pronounced in experimental acute subgroup than in experimental chronic subgroup. Decrease in thickness of granular layer was found in stressed rats of both groups but insignificant difference was found between their experimental and control subgroups.

DISCUSSION

This project had an objective to observe and compare the morphological changes in vermal cerebellar cortex of young male Sprague Dawley rats, when exposed to acute and chronic immobilization stress (CIS). From the study results, it was found that the animals of the experimental group I were affected more regarding their activity, interest in food intake, urination and defecation as compared to the experimental group II, when removed from their restrainer after continuous immobilization stress of 24 hours and 2 hours respectively. The animals of immobilized group I showed hypo activity more as compared to their immobilized group II. Loss of interest in food intake was found more in acutely stressed group as compared to chronic. Similarly the plastic bottles of the experimental animals of group I were filled more with urine and fecal matter as compared to experimental animals of group II. These behavioral changes in stressed rats could be due to the activation of autonomic nervous system, during which the noradrenergic activity is increased. Our results are in agreement with the results of Hellriegel and D'Mello[15]and Tanaka et al[16] who have reported time-related changes as also observed in our study in immobilization stress- induced

Table-I. Summary of results of comparative study of various thickness of Vermal cerebellar cortex parameters

Parameters	Acute immobilization "E" compared with control	Chronic immobilization "E" compared with control	Acute vs chronic
Urination	Increased Significant	Increased Non-significant	Non-Significant
Defecation	Increased Significant	Increased Non-significant	Non-Significant
Activity	Decreased Significant	No effect	Non-Significant
Interest in food intake	Decreased Significant	No effect	Non-Significant
Thickness of vermal cerebellar cortex (µm)	Increased Non Significant	Increased Non-significant	Decreased Non-Significant
Thickness of molecular layer (µm)	Increased Non Significant	Increased Non-Significant	Decreased Non-Significant
Thickness of granular layer (µm)	Decreased Non Significant	Decreased Non-significant	Decreased Non-Significant

"E" = Experimental, AIS = Acute immobilization stress, CIS = Chronic immobilization stress
** Significant *** Highly Significant*

enhancement of noradrenergic activity. This could be attributed to activation of the hypothalamus, amygdala, and thalamus.

Increase in thickness of vermal cerebellar cortex and molecular layer was found in our study that could be due to brain edema, which is in accordance with the previous studies, where Gheorghe D, et al[17] studied that acute stress also has pro-inflammatory effects that was seen in the diencephalon and cerebellum by significant increased permeability of the rat blood brain barrier to intravenous 99Technetium gluceptate (99Tc) but in an other study this effect was absent in the diencephalon and cerebellum of mast cell-deficient mice. Studies of Sharma et al[18] also support the involvement of blood-brain barrier permeability and 5-hydroxytryptamine (5-HT) level, which were increased in 12 out of 14 brain regions in young rats, on exposing them to immobilization stress for eight hours and for long term IS. Shanker et al[19] found increased plasma and brain 5-HT levels in various brain regions of young rats with increased BBB permeability after eight hours immobilization stress.

The results of our study are in agreement with the study results of Belova et al[20], in which they found disruption of blood brain barrier due to ruptures of a number of vessels and penetration of dye into the parenchyma of various brain areas in a fluorescence microscopic study in rats after acute immobilization stress (AIS). In our opinion and keeping in view the results of others, it is probably accumulation of fluid in the brain matter due to the leakage of blood brain barrier resulting in brain edemas which ultimately causes increase in thicknesses of various layers of the cerebellar cortex. It is seen that AIS causes more activation of energy metabolism as compared with CIS as observed by Deepak et al[21] in a study where they immobilized rats for 150 min, once only, and for 10 consecutive days in CIS, which is probably due to habituation of animals in prolonged stress. Their results are in accordance with results of our study where changes thicknesses of the cerebellar layers have been found more pronounced in AIS than in CIS. According to Bruno et al[22] induction of c-fos and CRF1 receptor transcripts in CRF-ir neurons of the Male Sprague-Dawley rat hypothalamus, exposed to a single 90-min immobilization stress or for 11 consecutive days was more in acute as compared with chronic.

Some people like Takami et al[23] say that IS causes increase in the intracellular Ca^2 level which results in induction of phosphorylation of calcium/calmodulin-dependent protein kinase (CaMK) II, is involved in the alteration of brain functions such as memory formation. They found significantly increased phospho-CaMKII levels in acute (single) and repeated (4 d), but not chronic (14 d), stress exposure of 45 min or longer duration. This shows that during the chronic stress the animals adapts themselves due to which the effects of this sort of stress was less pronounced as compared to acute which is in agreement to our study.

In a comparative study by Murielle et al[24] also found more alterations in cerebral protein synthesis in response to acute as compared with CIS in the rat which indicates the animal's ability to restore homeostasis during chronic stressful situations. Sheikh et al[25] studied that levels of serotonin, dopamine and norepinephrine in different parts of the brain were higher in acute immobilization (150 minutes one time only) than in chronic stress (seven days).Moreover they also noticed that treatment with Bacopa monniera (BM), levels of all three chemicals in one part of the brain were normalized in chronic stress while in acute only two were normalized, showing the adaptability of animals in chronic stress. Keeping in view the results of above studies, it can be said that the morphological changes in chronic immobilization stress although insignificant were less marked than acute, but they signify the process of adaptability in animals immobilized for chronic stress. Blood-brain barrier permeability and mast cell activation could be the cause of brain edema due to which increased thickness of vermal cerebellar cortex and molecular layer have been found, in this current study.

CONCLUSIONS

Our study results suggest that cerebellar cortical tissue is influenced by immobilization stress by which the out come of cerebellar activity may be reduced.

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"Success is not final,
Failure is not fatal,
It is the courage to Continue that counts."

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