Acta Scientific ANATOMY

Volume 3 Issue 1 January 2024

Research Article

Effect of Environmental Enrichment on Stress Mediated Changes in the Prefrontal Cortex of the Cerebrum of Male Wistar Rats

Samuel Chukwuebuka Onyealilam*, Simon Oche Onuh, Sunday Musa

Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

***Corresponding Author:** Samuel Chukwuebuka Onyealilam, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Received: December 28, 2023

Published: January 04, 2024

Abstract

Background of Study: Stress is unavoidable, acting as a precursor to physiological and behavioral changes, as well as supporting structural and functional changes linked to memory, behavioral control, and the developmental process, impacting on brain defect, resulting to poor working memory and attention flexibility.

Aim: The aim of the study is to investigate the effect of enriched environment on stress mediated changes in the prefrontal cortex of adult male Wistar rat.

Material and Methods: Thirty adults male Wistar rats weighing 150g were used and grouped into five with each group containing six rats each; Group I (control group), Group II (enriched only for 2hrs), Group III (stressed only for 2hrs), Group IV (stressed + enriched for 1hr), and Group V (stressed + enriched for 2hrs), their body weight and brain somatic index were recorded, and anxiety using open field apparatus were studied, light microscopy of the brain was also observed. Data was analyzed using statistical package for social sciences (IBM SPSS 20).

Results: The results from this present study showed a significant increase ($p < 0.05$) on the final body weight across all groups when compared to their initial also, there is a decrease in body weight of stressed alone group across all groups when compared to others groups. However, there was a decrease in the cerebrum weight of stressed alone group when compared to other groups, and there was also an increase in the stress + enriched one hour and stress + enriched two hours when compared to the stress alone group, but this was not significant ($p < 0.005$).

Conclusion: Chronic stress in Wistar rats can cause reduction in food intake, have no significant changes on body weight, hamper on cognitive and executive activities of the brain, following the effects on anxiety related behavior. With respect to the findings of present result, exposure to an enriched environment will improve learning and memory, enhance healthy executive functions of the cerebral cortex.

Keywords: Cerebrum; Prefrontal Cortex; Human Anatomy

Introduction

Stress is a well-known phenomenon that surrounds human lifestyles, a sensation that has been well-understood throughout human history and a characteristic of living that is heavily stressed [1]. Low levels of it may have a health advantage, boosting biopsychological health and performance, but a rise in its level may cause biopsychosocial problems and even significant harm to individuals, according to psychological sciences [2].

Stress is unavoidable, acting as a precursor to physiological and behavioral changes, as well as supporting structural and functional changes linked to memory, behavioral control, and the developmental process. Chronic or exceptionally high levels of stress (toxic stress), on the other hand, have been proven to have detrimental effects on the brain's neural network [3,4]. The time of stress exposure is just as important as the amount of stress, with early stress exposure having a particularly powerful and long-lasting effect [5].

The benefits of enriched animals may extend beyond those related to the restoration of normal living conditions in the typically impoverished lab rat. Consorti *et al.* [6] researchers found that the social grouping of rats was insufficient to improve cognition, and that the availability of inanimate objects was crucial [7]. There may be a dose response curve associated with enrichment in caged animals: too little, and boredom sets in; just the right amount, and creativity and engagement flourish; too much, and overstimulation or habituation set in. Environmental enrichment promotes synaptogenesis (the creation of synapses) and neuron survival during early growth [8]. The influence of enriched environments on the development of the visual system has been the subject of the most research. Enriched environments, for example, have been shown to boost brain-derived neurotrophic factor (BDNF), a protein that stimulates neuron growth and maturation.

Material and Method

Experimental animals

Thirty (30) adult Wistar rats weighing between 120-160g were purchased from the animal house of the Department of Human Anatomy, Faculty of Medical Basic Science, College of Medical Sciences, Ahmadu Bello University, Zaria-Nigeria. After acquisition of the rats, they were housed in standard rat cages and allowed access to standard pelletized diet and water *ad libitum.* They were maintained in this condition for seven (7) days to acclimatize them prior to commencement experimental study.

Stress apparatus

The cages were constructed using 25mm diameter × 11mm depth transparent acrylic tubing, covered on opposing sides and barrier between the tubing, creating 5 tubes in one restrain cage according to MacGillivray and Anderson [9], made from the Department of Human Anatomy, Ahmadu Bello University Zaria, Nigeria.

Enrichment Platform

Three (3) large enrichment platform with dimension (80cm × 40cm × 56cm) were made, plywood as the frame and transparent plastic as the base, with each platform containing variety of objects such as curved plastics, wooden ladder, tennis balls of different colors, balloons, plastic covers of different colors, etc [10].

Other materials

There following materials were also used; digital weighing balance, cages for housing, water bottles, sawdust for bedding, distilled water, dissecting kits, dissecting board, razor blade, mortar and pestle for homogenate, syringes (2ml and 5ml), cotton wool, plain and EDTA bottles for blood collections, anesthesia (chloroform), Phosphate Buffered Solution (PBS), ice block used to keep the blood, homogenate, PBS cool.

Animal sacrifice

After the last day of the experiment, at about 24 hours later, the animals were sacrificed using chloroform anesthesia, and the organs of interest (the brain; cerebrum) was harvested and fixed in 10 % Formal saline, after measuring the weight, using the weighing balance. This procedure was done according to the ethical approval obtained.

Determination of weight of Wistar rats

On the days of experimentation, the animals' weight was taken using a digital weighing balance and the readings were documented.

Brain weight

Immediately after excision of the brains from the skull they were quickly weighed using a sensitive weighing balance with sensitivity of 0.01g. After weighing the brain, the resultant weights were recorded.

Brain somatic index estimation

The whole brain was weighed individually using a sensitive digital weighing balance. The brain somatic index was calculated from the formula below

$$\frac{\text{Brain weight (g) i}}{\text{Final body weight (g)}} \times 100$$

Oxidative stress markers

The resultant supernatant was stored in plain bottles for estimation of oxidative stress markers at the Department of Human Anatomy, Ahmadu Bello University Zaria, Nigeria.

Superoxide dismutase activity

This was determined according to the method of Fridovich [11], this assay is based on the ability of superoxide dismutase to inhibit auto oxidation of adrenaline at pH 10.2.

Lipid peroxidation (Malondialdehyde)

Lipid peroxidation was determined according to the method of Ohkawa., *et al.* [12], generates peroxide intermediates which upon cleavage release malondialdehyde, a product that reacts with thiobarbituric acid. Therefore, it is determined based on thiobarbituric-acid reactive substance (TBARS).

Assessment of reduced glutathione

This was determined according to the method of Ellman [13]. The principle of assay is based on the reaction of 5, 5'-dithiobis nitrobenzoic acid (DNTB) and reduced glutathione (GSH).

Histology and histochemistry

The brain was separated divided into two halves, one part for homogenate and the other part was weighed and immediately fixed in neural formosalin. It was then processed [14], dehydration [15], clearing [16], and infiltration [17] routinely, embedded in paraffin wax and sectioned at 10um thickness prior to staining using H &E [18], the tissues were then mounted with cover slips using a mounting media.

Stress Induction

Restraint stress was performed 2hrs/day for Group C, D, and E as described by Sántha., *et al.* [19], the procedure is being used to denote both physical and physiological stress.

Enrichment protocol

Three large boxes were constructed with plywood as its frames and transparent plastics as its base, sanitized 'with ethanol, a wooden ladder, hand-cut plastics to form tunnels, and other material to elicit fun for the animal were also sanitized with ethanol. Saw dust was used as bedding, and the fun eliciting materials were arranged at different locations inside the box. After setting the enriched environment, the rats in Groups II, IV, and V were introduced into the environment and this procedure was carried out for four (4) weeks, with the environment setup being changed daily.

Experimental design

Adult male Wistar rats were randomly distributed into five (5) experimental groups of 6 animals each, using a Random Allocation Software developed by Saghaei [20]. Each rat in a group were marked on their tails based on randomization by the software; Group A served as Control and were served with pelletized food, Group B were enriched for 2hrs (2pm-4pm) with food and water *ad libitum,* without stress, Group C were stressed for 2hrs (9am-11am) without enrichment, Group D and E were stressed for 2hrs (9am-11am) and were also enriched but with different timing: Group D were enriched for 1hr while Group E were enriched for 2 hrs.

Photomicrography

After staining with H and E, slides were viewed under the microscope and photomicrographs were taken using, ×250 magnifications in five (5) fields respectively, using MD900 Amscope digital camera.

Data Analysis

Data obtained from the study were expressed as Mean±SEM. One way analysis of variance (ANOVA) was used to compare the mean differences between and within the groups followed by LSD *post-hoc* test. *p*-value less than 0.05 was considered statistically significant. Data was analyzed using statistical package for social sciences (IBM SPSS 20).

Results

Body weight

As seen in Figure 1, on the assessment of the effect of environmental enrichment following exposure to stress on adult male Wistar rat, there was a significant increase ($p < 0.05$) on the final body weight across all groups when compared to their initial. Also, there is a decrease in body weight of stressed alone group across all groups as shown in figure 2 when compared to others groups, but this was not significant ($p < 0.05$).

From the result as shown in figure 2, there was no significant difference across all groups, although, there was a decrease in the percentage weight change of the stressed alone group when compared to other groups, an increase in percentage weight change for the stressed with enriched one, and stressed with enriched two hours ($p < 0.05$).

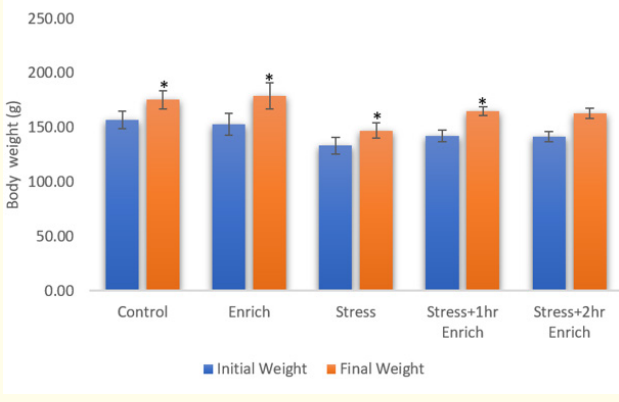

Figure 1: Initial and final body weight of Wistar rats of the entire group for the whole weeks.
n = 5; mean ± SEM, paired sample t-test, *= p < 0.05 when initial weight is compared to final weight.

Brain-somatic index

From the result as shown in figure 3 there was no significant different across all groups in the cerebrum weight, however there was a decrease in the cerebrum weight of stressed alone group when compared to other groups, and there was also an increase in the

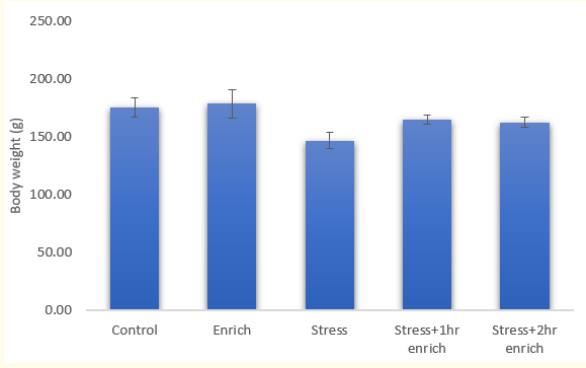

Figure 2: Final body weight of Wistar rats for the entire group. n = 5; mean ± SEM, one-way ANOVA.

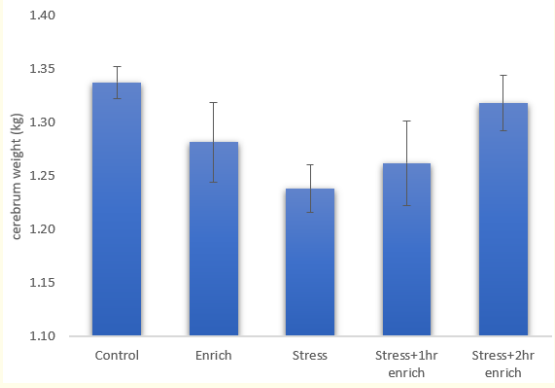

Figure 3: Cerebrum weight of experimental Wistar rats of the entire group.
N = 5; mean ± SEM, one-way ANOVA.

stress + enriched one hour and stress + enriched two hours when compared to the stress alone group, but this was not significant ($p < 0.005$).

Anxiety using open field test apparatus.

Figure 4 showed the level of anxiety recorded from the frequency of bolus, a neurobehavioral study using the Open Field Apparatus, within four weeks of the experiment for the five groups. There was no significant difference ($p < 0.05$) when compared throughout the entire group, but an increase in anxiety level was noted from the stressed + enriched 1hr group when compared with the stressed alone group at the fourth week. Also, an increase in anxiety for the stressed + enriched 2hrs when compared with the stressed alone group and the control at the third week ($p < 0.005$).

From the result seen in figure 5, there was no significant difference noted in the frequency of urine, but an increase was noted at the third week with stressed + enriched 1hr when compared with stressed alone group. Also, there was an increase in urine frequency for the control group when compared with the stressed alone group ($p < 0.005$).

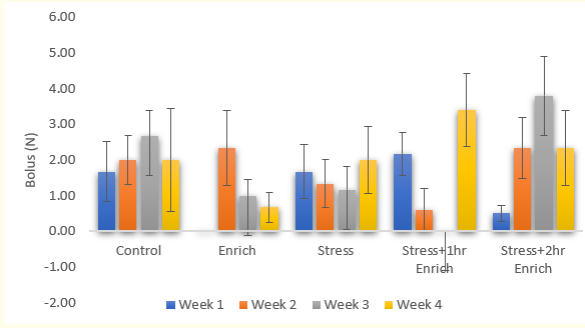

Figure 4: Frequency of bolus in open field test taken for the entire group.
Mean ± SEM, split-plot ANOVA.

Oxidative bio-makers (SOD, GSH, and MDA) using chemical method

As seen in Table 1 below, Superoxide dismutase (SOD), Glutathione (GSH) and Malondialdehyde (MDA), which are markers for lipid peroxidation were estimated and it was found that there was a significant increase of the MDA mark in stressed alone group (Group III) when compared with the enriched alone group for 2hrs (Group II) and control group (Group I) ($p < 0.05$). Also, still on the MDA marker, there is a change in reduction between stressed + enriched 1hr (Group IV) and stressed + enriched 2hrs (Group V) but no significant difference ($p < 0.05$).

From the table below, there is a noticeable decrease in the antioxidant maker GSH from control (Group I) to stressed alone group (Group III), but an increase from stressed + enriched 1hr (Group IV) to stressed + enriched 2hrs (Group V) but no significant difference ($p < 0.05$). Also, for SOD antioxidant maker is seen to be increasing across the group from (Group I) control to (Group III) stressed alone group, and a slight decrease is seen from (Group IV) stressed + enriched 1hr to (Group V) stressed + enriched 2hrs but no significant difference.

Discussion

Long-term stress causes the loss of dendrites and spines in the PFC, according to animal studies [21,22]. The loss of dendrites is linked to poor working memory and attention flexibility [23]. The present study was to assay the effect of enriched environment over stress in the prefrontal cortex of the cerebrum of adult male Wistar rats.

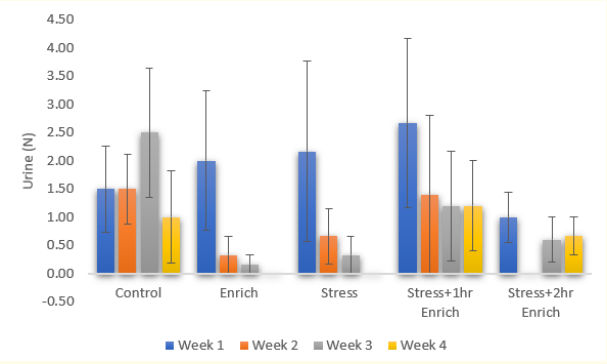

Figure 5: Frequency of urine in open field test taken for the entire group.
Mean ± SEM, split plot ANOVA.

In this study, there was a significant increase in final weight across all the groups when compared together, taking from initial weight (weight before the commencement of the experiment) and final weight (before the animals were sacrificed). This might possibly be a result of stress hormone, where Chronic stress generally promotes wanting, seeking, and intake of palatable high-fat and energy-dense foods [24].

Results obtained indicates that chronic stress can contribute to the development and/or maintenance of mental health problems *because* of the capacity of stress hormones (cortisol in humans and corticosteroids in rodents) to access the brain and impact cognitive and/or affective processing [25]. Studies have shown that stress hormones can lead to impairments in attention, memory, and emotion processing in humans [26,27].

Conclusion

In the present study, histo-morphological, biochemical and neurobehavioral evaluation of the prefrontal cortex following the administration of stress and exposure to enriched environment in Wistar rats. The results from the present study show that chronic stress in Wistar rats can cause reduction in food intake, have no significant changes on body weight, hamper on cognitive and executive activities of the brain, following the effects on anxiety related behavior. With respect to the findings of present result, exposure to an enriched environment will improve learning and memory, enhance healthy executive functions of the cerebral cortex.

Bibliography

1. De Raeve L., *et al*. "Mental health effects of changes in psychosocial work characteristics: a prospective cohort study". *Journal of Occupational and Environmental Medicine* 49.8 (2007): 890-899.

2. Tse JLM., *et al*. "Facets of job effort in bus driver health: Deconstructing" effort" in the effort-reward imbalance model". *Journal of Occupational Health Psychology* 12.1 (2007): 48.

3. Drury SS., *et al*. "Shaping long-term primate development: Telomere length trajectory as an indicator of early maternal maltreatment and predictor of future physiologic regulation". *Development and Psychopathology* 29.5 (2017): 1539.

4. Ra R and Androulakis IP. "Allostatic adaptation and personalized physiological trade-offs in the circadian regulation of the HPA axis: A mathematical modeling approach". *Scientific Reports* 9.1 (2019): 1-15.

5. Hegde A., *et al*. "Early-life short-term environmental enrichment counteracts the effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala". *Scientific Reports* 10.1 (2020): 1-13.

6. Consorti A., *et al*. "From basic visual science to neurodevelopmental disorders: the voyage of environmental enrichment-like stimulation. *Neural plasticity* (2019).

7. Cué CM and Dierssen M. "Plasticity as a therapeutic target for improving cognition and behavior in Down syndrome". In *Progress in Brain Research* 251 (2020): 269-302.

8. Morichetto H and Nilsson M. "Can Residential Architecture Constitute a Part of a Human-Enriched Environment and Contribute to Recovery, Prevention and Stress Reduction?1". In *Architecture for Residential Care and Ageing Communities* (2020): 53-64.

9. MacGillivray M and Anderson G. "Three Useful Insect Cages1". *The Canadian Entomologist* 89.1 (1957): 43-46.

10. Slater AM and Cao L. "A protocol for housing mice in an enriched environment". *JoVE (Journal of Visualized Experiments)* 100 (2015): e52874.

11. Fridovich I. "Superoxide dismutases: an adaptation to a paramagnetic gas. *Journal of Biological Chemistry* 264.14 (1989): 7761-7764.

12. Ohkawa H., *et al*. "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". *Analytical Biochemistry* 95.2 (1979): 351-358.

13. Ellman GL. "Tissue sulfhydryl groups". *Archives of Biochemistry and Biophysics* 82.1 (1959): 70-77.

14. Feldman AT and Wolfe D. "Tissue processing and hematoxylin and eosin staining". In *Histopathology* Springer (2014): 31-43.

15. Rylander CG., *et al*. "Dehydration mechanism of optical clearing in tissue". *Journal of Biomedical Optics* 11.4 (2006): 041117.

16. Jing D., *et al*. "Tissue clearing of both hard and soft tissue organs with the PEGASOS method". *Cell Research* 28.8 (2018): 803-818.

17. Kato M., *et al*. "Eosinophil infiltration and degranulation in normal human tissue". *The Anatomical Record: An Official Publication of the American Association of Anatomists* 252.3 (1998): 418-425.

18. Bederson JB., *et al*. "Evaluation of 2, 3, 5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats". *Stroke* 17.6 (1986): 1304-1308.

19. Sántha P., *et al*. "Restraint stress in rats alters gene transcription and protein translation in the hippocampus". *Neurochemical Research* 37.5 (2012): 958-964.

20. Saghaei M. "An overview of randomization and minimization programs for randomized clinical trials". *Journal of Medical Signals and Sensors* 1.1 (): 55.

21. Baratta MV., *et al*. "Controllable stress elicits circuit-specific patterns of prefrontal plasticity in males, but not females". *Brain Structure and Function* 224.5 (2019): 1831-1843.

22. Moench KM., *et al*. "Prior stress followed by a novel stress challenge results in sex-specific deficits in behavioral flexibility and changes in gene expression in rat medial prefrontal cortex". *Hormones and Behavior* 117 (2020): 104615.

23. Shu Y and Xu T. "Chronic social defeat stress modulates dendritic spines structural plasticity in adult mouse frontal association cortex". *Neural Plasticity* (2017).

24. Masih T., *et al*. "Stress-induced eating and the relaxation response as a potential antidote: A review and hypothesis". *Appetite* 118 (2017): 136-143.

25. Piccolo LdR., *et al*. "Can reactivity to stress and family environment explain memory and executive function performance in early and middle childhood?" *TRENDS in Psychiatry and Psychotherapy* 38 (2016): 80-89.

26. Baudin C., *et al*. "Saliva cortisol in relation to aircraft noise exposure: pooled-analysis results from seven European countries". *Environmental Health* 18.1 (2019): 1-12.

27. Lupien SJ., *et al*. "The effects of chronic stress on the human brain: From neurotoxicity, to vulnerability, to opportunity". *Frontiers in Neuroendocrinology* 49 (2018): 91-105.