Neurodegeneration of Pyramidal Cells and Memory Impairment in the Hippocampus of the Brain of Wistar Rats Exposed to X-Ray

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***Abstract*: This study investigated the histological changes in the hippocampus of the brain in rats exposed to radiation using a plain x-ray. A total of 20 rats were used for the experiment. The rats were divided into four (4) groups (n=5). Group A is the control group while groups B – D were exposed to radiation (255kv) for a specific duration. Water Morris maze was used to assess the neurobehavioral changes in memory and learning. Histological procedures were carried out to see the changes in the histology of the hippocampus. Results show that there was a significant difference in the time taken for the rats to reach the cemented block between the four groups ($F(3, 8) = 6.926$, $p<0.013$). Post-hoc analysis using the Bonferroni methods further revealed that the time taken to reach the cemented block for rats in Group D (42.67±10.50 secs) was significantly different from rats in Group A (7.67±2.52 secs). This is also an indication of loss of memory. Histologically it was observed that there was degeneration of pyramidal cells in the hippocampus of the brain. Degenerating neurons were also observed. X-radiation has been shown to affect memory n this experiment.**

***Keywords*: Hippocampus, pyramidal cells, exposed adult rats, brain, water Morris maze, radiation, degenerative neurons, cornu Ammonis, Neural progenitor cells, Dentate gyrus.**

I. INTRODUCTION

The hippocampus is a part of the brain which belongs to the limbic system. It lies under the medial temporal lobe, one on each side of the brain. The hippocampus is very important for the formation of new autobiographical and fact memory traces. It can be said to function as a memory gateway through which new memories must pass before being stored permanently in the brain. Bilateral hippocampal damage can result in anterograde amnesia i.e. loss of ability to form new memories, although older memories may be safe. Hippocampus proper is also known as Cornu Ammonis (CA), which consists of CA1, CA2, CA3, and CA4 sub-regions (Afeefy *et al*., 2013).

The health benefits of the use of radiation in the medical field are currently applauded but as the saying goes that everything that has an advantage has a disadvantage. The use or exposure of an individual to ionizing radiation (X-ray) also has adverse effect which ranges from instant cell death, reproductive death, mitotic or genetic death, chromosome breakage, interference of function, etc. a good example of the adverse effect of ionizing radiation is the risk of pregnancy, especially for their embryo to develop structural and functional defects. These observations are specifically relevant in cases of radiotherapy treatment or medical imaging during pregnancy which often results in abortion, delay of maternal therapy, or preterm delivery when women were diagnosed with cancer during delivery (Han *et. al*., 2016).

The human body is made up of different types of cells and tissues which vary in their degree of radio sensitivity and the human brain is not an exception. The brain is strongly sensitive to ionizing radiation which is the exact case when exposure interferes with a particular development time that is between six (6) to twenty (20) weeks of gestation (Otake *et al*., 2016).

Goldstein and Murphy (2012), reported on mental retardation and microcephaly resulting from prenatal radiation exposure as revealed in 38 cases of children born to mothers that received pelvic radiotherapy. Much after, this awareness was further strengthened and quantitative data were provided through the follow-up of the health of atomic bomb health survivors, primarily performed and published (Otake *et al*., 2016). Their study involved 1500 individuals exposed in utero to the radioactive fallout of the atomic bomb in Hiroshima and Nagasaki. A higher occurrence of generalized growth retardation and microcephaly, mental disability, seizures as well as a decreased school performance and scoring on an intelligence test. In all, it is evident that above a certain threshold, irradiation during the gestational period hampers normal brain development and functioning during later life when exposure occurred during the peak of neural expansion and differentiation.

In search for abnormalities that occur immediately after prenatal radiation exposure and that might account for a prolonged cognitive effect as seen in human cohorts, animal studies have been proven highly valuable (Verheyde *et al*., 2007). The rodent brain is a widely and acceptable used model system, given the obvious advantages of working with small

rodents. It has a high similarity in brain development architecture and interconnectivity when compared to humans.

Studies have shown that damage to the hippocampal neurons may lead to impairment of memory and learning, behavioral disturbances, and impact on Hypothalamo-Pituitary-Adrenal (HPA) axis (Daniels *et al*., 2009), (Narayanan *et al*., 2010), (Bolla S. R., 2015). The present study was undertaken to evaluate the short-term exposure effect of X-ray on cognitive functions like spatial learning, working memory, and hippocampal morphology in the albino rat.

II. MATERIALS AND METHODS

The research is an animal experimental study. The animal was acquired from the animal house, Department of Pharmacology, University of Port Harcourt, and allowed to acclimatize for two weeks. The animals were fed with rat feed and water. The animals were divided into four groups with five (5) rats in each group. Group A was the control group, group B was exposed for four (4) days, group C was exposed for nine (9) days and group D was exposed for fourteen days (2 weeks).

The rats were sacrificed at the end of each group experiment and the spinal cords were extracted and fixed in 10% formalin for 24hrs before a histological study was carried out on them.

Anesthesia was achieved by inhalation of anesthesia containing 0.001percent of diphenylamine which resulted in mild anesthesia. 5ml of anesthetic was used to soak sterile cotton wool balls and the balls were placed into a glass desiccator. The rats were placed individually into the desiccator and they were thoroughly monitored until they are anesthetized.

Radiation Technique

The amount of time for exposure of the test animals was two weeks; the amount of X-radiation dose was constant across all the groups except for the control group which was not radiated at all. The difference among the group was the total number of days used for exposure; a mobile x-ray machine was used. The anesthetized rat was placed centrally on a bench and the distance between the animal and the x-ray machine was 10cm. The centering point of the x-ray were focused mainly on the head [brain] and spinal cord.

Figure 1. An exposed rat during the experiment.

Group A: This was the control group and no radiation was administered to this group.

Group B: was exposed to radiation for four days and sacrificed for histology.

GROUP C: was exposed to radiation for nine days and sacrificed for histology

Group D: was exposed to radiation for fourteen days and sacrificed for histology.

Exposure Factor and time: The exposure factor and time used on the rats are 85kv and 20MAS. Each rat is exposed to the radiation three times making a total of 255kv and 60MAS per day.

Behavioral Experiment

Water Morris Maze was used to assess the spatial learning and cognitive function of the rats. A Morris water maze was carried out whereby we used a divided tank of about 6 feet in diameter and 2 feet in depth and an escape platform [cemented blocks] was placed at the center of the tank one inch above the water. The rat was dipped carefully into a 26-degree temperature of water whereby they swim round the edges of the tank for about 60 seconds before removing them. It was observed they could not locate the escape platform at first so they were trained to always swim toward the block for safety. The animal is placed at the first specified drop location about 20cm away from the safe zone. The time rate begins immediately after the animal is dropped into the water and ends when the animal rests on the platform [cemented blocks]. For the animals who could not locate the platform in 60 seconds, we assisted them to the platforms and allowed them to settle for about 15 seconds before putting them into their cages.

Organ Harvesting

The rats were sacrificed humanly, the brain was harvested and fixed in 10% formalin for 24 hours before subsequent tissue processing.

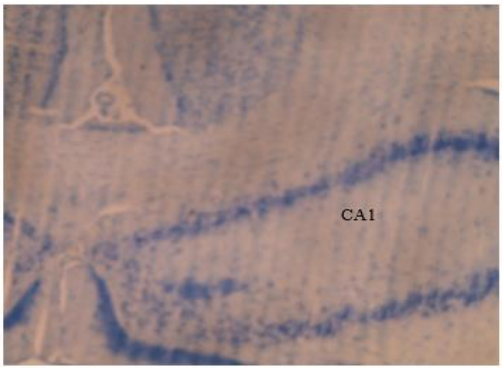
Data Analysis

The results were expressed in Mean±SD and analysed by using one-way ANOVA to check the level of significance among groups followed by an ANOVA post-hoc test (Bonferroni correction). The $p<0.05$ was considered as statistically significant.

Histological Procedure

The brain was fixed in formalin, dehydrated with alcohol; it was cleared with xylene and infiltrated with paraffin wax. The tissue got embedded and thin sections were cut using a microtome. H and E samples were carried out on the sections to show the cytoarchitecture (microscopic structure). The slides were viewed under a light microscope.

III. RESULTS



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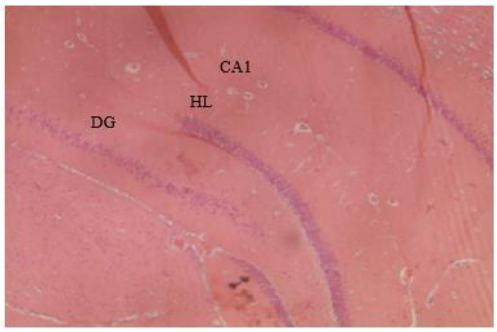

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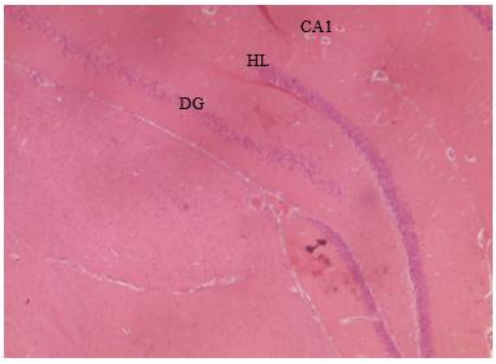

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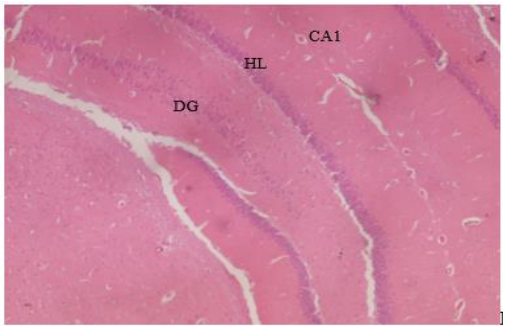

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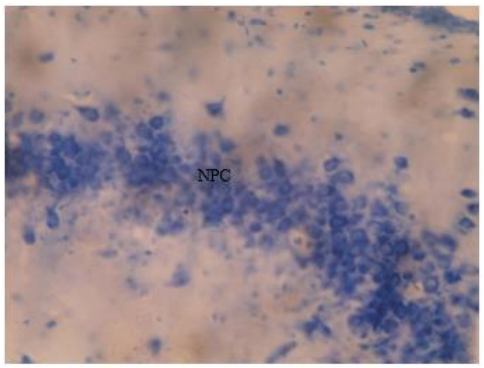
Figures 2. Shows the photomicrograph of 100x magnification of (A) control rats (B) Cytoarchitecture of the hippocampus in 3 days X-ray exposed adult rats (C) Cytoarchitecture of the hippocampus in 7 days X-ray exposed adult rats (D) Cytoarchitecture of the hippocampus in 14days X-ray exposed adult rats. CA1- cornu Ammonis region 1, DG-dentate gyrus.



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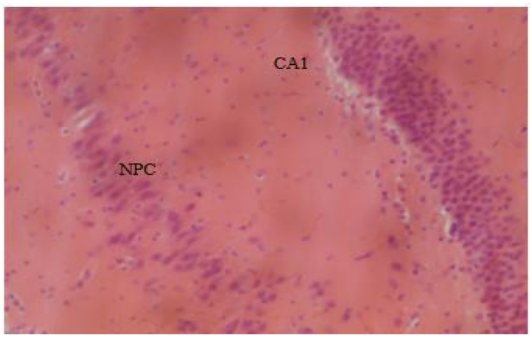

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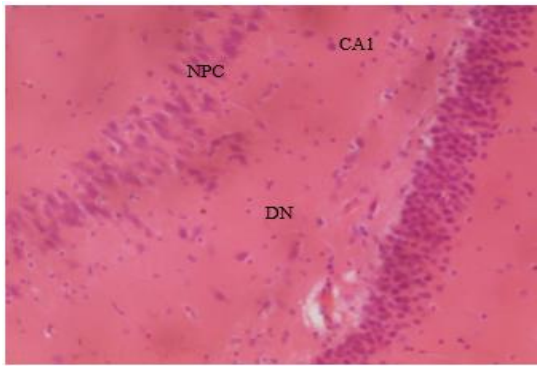

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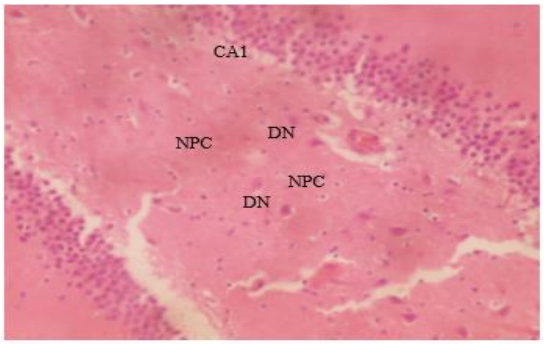

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Figures 3. Shows the photomicrograph of (A) control rats and (B) Hippocampal pyramidal cell layers in 3 days X-ray exposed adult rats, 400x magnifications. The pyramidal cells of the hippocampal layer are normal in 3days x-ray exposed rats. (C) Hippocampal pyramidal cell layers in 7 days X-ray exposed adult rats, 400x magnifications. Few degenerated pyramidal cells

are seen (D) Hippocampal pyramidal cell layers in 14 days X-ray exposed adult rats, 400x magnifications. Degeneration of pyramidal cells was observed in 14 days x-ray exposed rats. Degenerative neurons (DNS) as well degenerative layers are also observed in these rats. NPC- Neural progenitor cells, CA1- cornu Ammonis region 1, DN- degenerative neurons.

Behavioural Studies

Table 1 shows results for behavioral studies using water Morris Maze.

Duration	Time Spent Navigating(s)			
	A	B	C	D
3days	10.00±2.52	13.00±7.77	17.00±14	32.00±10.50
7days	8.00±2.52	17.00±7.77	21.00±14	43.00±10.50
14days	5.00±2.52	28.00±7.77	43.00±14	53.00±10.50

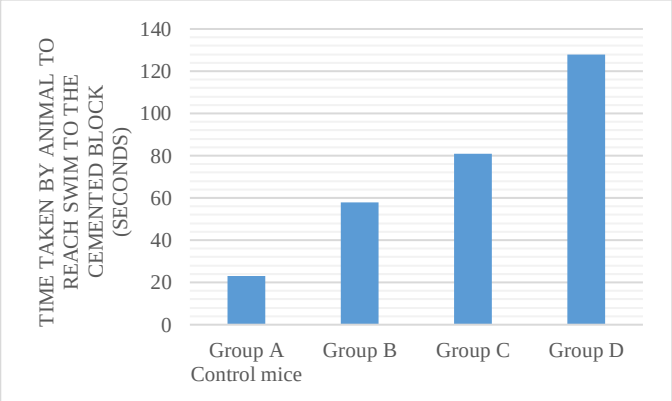

Fig. 4. Shows results for Water Morris Maze

IV. DISCUSSION

The time taken by the rats to reach the cemented blocks immediately after it has been put into the water was significantly increased in group B (58 seconds), group C (81 seconds) and group D (128 seconds) compared to group A (control group).

The time taken by the animal to reach the cemented block scores in Group A vs Group B (7.67±2.52 vs 19.33±7.77), was not significant (p>0.01); Group B vs Group C (19.33±7.77 vs 27±14), was not significant (p>0.01); Group C vs Group D (27±14 vs 42.67±10.50), was not significant (p>0.01); Group D vs Group A (42.67±10.50 vs 7.67±2.52 seconds), was statistically significant (p<0.01). This also an indication to loss of memory. [Fig-4].

On histological examination, degeneration of the pyramidal cells, degenerative neurons, and degeneration layers were observed after fourteen days of X-radiation exposure. Also, the pyramidal layers were deprecated which cause a general weakness in the exposed rats and as such could not control their voluntary muscular movements. In humans, it causes lower facial weakness and changes in speech. Monje *et al.* (2002) reported at in the absence of radiation proliferating cells in the SV2 and SG2 develop into new neurons with appreciated efficiency of 80%. This study is similar to Bolla S. R (2015) who reported that exposure to 800 MHz mobile radiation for 30 days led to increased neuronal damage and decreased viable neurons in the hippocampal CA3 region.

Miwa *et al* (2016) discovered that radiation decreased the brain volume, increased ventricle volume, and reduced cortical and white matter thickness in irradiated rats when assessed using Magnetic Resonance Imaging (MRI). They also discovered that rats that were irradiated at embryonic day 15 exhibited postnatal changes such as thinner cerebral cortex, decreased size of the whole brain, and increased ventricle volume.

However, no abnormalities in the cortical 6-layered structure were observed in any of the radiation-exposed rats. Nevertheless, ectopic neurons were observed only in the hippocampus (beneath the cerebral cortex) of rats exposed to 255kv of radiation prenatally. Sorrels et al., (2018) concluded that neurogenesis does not continue in the human adult hippocampus, or is extremely rare. Regardless, their study also reminds us that simple translation of results from animal studies to human studies may be problematic. On the contrary, hippocampal neurogenesis was demonstrated in aging adults and defected in patients with mild cognitive impairments and Alzheimer's disease (Tobin, 2019). He also provided evidence that the extent of neurogenesis, particularly the number of newly forming neurons, is associated with a better cognitive diagnosis.

Although, most research work carried out, only measured the absorbed dose which is in gray (Gy), we would be comparing with the exposure factor used for our X-ray machine which is in Kilovolt (KV). The effects of X-radiation doses below 10 GY on the rodent brain have been widely studied (Monje *et al*., 2002) as a model system for damage incurred from radiotherapy for brain tumors and also as a way of interrupting neurogenesis. In the absence of radiation, proliferating cells in the SVZ and SGZ develop into new neurons with appropriate efficiency of 80% (Monje et al., 2002). Delivery of radiation at doses as low as 2Gy has been shown to suppress cellular proliferation in both SVZ and SGZ (Tada et al., 2000).

After much observation and analysis, it was observed that the unexposed rats became very conversant with the escape platforms i.e the more they swim, the faster they arrived at their escape platforms. This was due to their un-exposure to X-radiation. Nevertheless, for the exposed rats, it was observed that there has been an increase in the loss of memory due to the induced radiation on them. The rats exposed to X-radiation for four days, nine days and fourteen days found it difficult to locate their escape platforms on time. As the day went by we noticed a continuous loss of memory. This is similar to the study carried out by Krishna *et al* (2019) while studying the effect of electromagnetic radiation and hippocampal morphology in Swiss albino mice. They found out that there was a loss of memory in rats exposed to radiation (both for the periods of 30 minutes/day and 60minutes/day) when compared to rats that were not exposed to radiation as the rats exposed to radiation took a longer time to locate the reward chamber than those that were not exposed to radiation. Nittby *et al*., (2008)

also reported that exposure to 900 GSM radiation will reduce memory functions in rats.

V. CONCLUSION

The use of X-radiation in the medical field is of great importance but it must be utilized with good caution since radiation exposure can evoke serious health consequences, as pointed out in hippocampal exposure in this work. The use of radiation excessively could degenerate pyramidal cells and layers and as such, doses of radiation-induced on individuals should be calculated and the ALARA (as low as reasonable achievable) principle must be strictly adhered to.

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