



**HISTOLOGICAL CHANGES IN THE HIPPOCAMPUS AND BEHAVIOR PATTERN
FOLLOWING EXPOSURE TO PAINT FUMES ON ADULT MALE WISATR RATS**

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Article Received on 16/03/2016

Article Revised on 04/04/2016

Article Accepted on 25/04/2016

ABSTRACT

People get exposed to paint fumes either as a result of remodeling of homes, nature of occupation and otherwise. Paint fumes are gaseous emissions from paints that get mixed up with the air around us and they contain volatile organic compounds which are known to be hazardous to human health. This study accessed the effect of paint fume exposure on 12 adult male wistar rats divided into 4 groups with 3 rats each. Group 1, 2 and 3 were exposed for 2, 3, and 4 weeks respectively while group 4 was the control group. At the end of each week, open field test was conducted to access some behavioral pattern in the rats. Animals were sacrificed using cervical dislocation, the brain dissected and the hippocampus was fixed, processed using routine processing schedule, paraffin sections were stained with H & E staining technique. Result showed in no observable cellular change in group 1 and group 4, while group 2 showed slight cellular changes in group 2 and marked degenerative changes in group 3. This duration dependent changes in the hippocampus may be as a result of prolonged exposure to paint fumes. Weight showed no marked and statistically variation across all groups, open field test revealed reduced locomotion, exploratory and increased anxiety across the exposed groups when compared with the control. This researched showed that exposure to paint fume caused degenerative changes in the hippocampus which may lead to amnesia and can also be anxiogenic. The public should beware and employ protective measures

KEYWORDS: Hippocampus, Paint Fumes, Open Field Test, Histology, Vocs, Wistar Rat.

INTRODUCTION

Paint fumes are gaseous emissions from paints that get mixed up with the air around us. Paints are made up of harmful chemicals such as solvents and volatile organic compound (VOCs) (Kesselmier and Staudt, 1999). VOCs have a high vapour pressure at room temperature and they are known to be the major indoor air pollutants at room temperature (Behr and Johnen, 2009). Paints are used on daily basis around the world either during remodeling of homes and offices or spraying of cars and individuals are always in contact with these paint fumes. A long term exposure to paint has been shown to be detrimental to human health as it causes sick building syndrome (SBS) (Wang *et al.*, 2009).

The brain is the most important and complex part of the body as it is the seat of intelligence, interpreter of senses, initiator of body movement and controller of behavior (Richard, 2006). It is made up of many parts but they all work together despite each part having its own specialty; he done of such part is the hippocampus (Richard, 2006). The hippocampus is part of the midbrain, it is located in the diencephalon of the cerebrum and it is involved in memory forming, organizing as well as storage (Richard, 2006). Particularly, it is important in forming new

memories and connecting emotions and senses such as smell and sound to memories (Amaral, 2006). A damage to the hippocampus can result in anterograde and reterograde amnesia (Catherine, 2006).

The open field test is a simple senseriomotor test used to determine the general activity and exploration habits on rodent models of central nervous system disorders. It measures both qualitative and quantitative general locomotor activity and willingness to explore in rats (Stanford, 2015). This present investigation investigated the histological effect of paint fumes on the hippocampus and its effect on behavioral patterns using adult male wistar rats.

METHODOLOGY

PROCUREMENT OF PAINT Choice emulsion paint was gotten from a local paint shop in Abraka, delta state although the content of the paint was not given.

ANIMAL HANDLING 12 adult male wistar rats were gotten from the animal house of the faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. They weigh between 145kg-190kg and were kept in standard conditions. The rats were given normal

rat feed and water and were kept to acclimatized for 13 days. After every 7 days of exposure to paint fumes, the rats were kept in an open field box for 10 minutes, after which they were taken back to their cages.

ANIMAL GROUPING

After 13 days of acclimatization, the rats were divided into 4 groups of 3 rats each;

Group 1: the rats in this group were exposed to 150ml of paint for 2 weeks, 8 hours daily

Group 2: the rats in this group were exposed to 150ml of paint fumes for 3 weeks, 8 hours daily

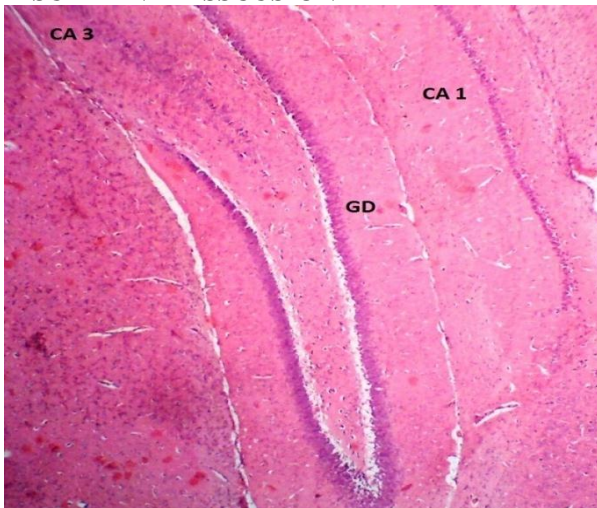
Group 3: the rats in this group were exposed to 150ml of paint fumes for 4 weeks, 8 hours daily

Group 4: the rats in this group were the control specimen; they were not exposed to paint fumes.

METHOD OF EXPOSURE The rats were placed in a chamber with little ventilation and after the period of exposure daily, they were taken back to their cages. On the last day of exposure, the rats were starved and after the period of exposure, they were sacrificed using cervical dislocation.

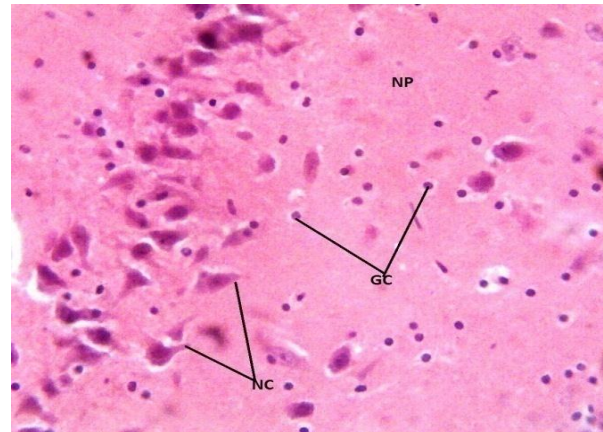
HISTOLOGICAL TREATMENT The hippocampus was fixed in Bouin's fluid, dehydrated in ascending grade of alcohol, cleared in xylene, impregnated and embedded in paraffin wax. They were sectioned and stained with H&E staining technique following Ekundina and Eze (2015) recommendation to minimize artifact. The images were captured with a digital microscope with a magnification of x100 and x400.

RESULT AND DISCUSSION



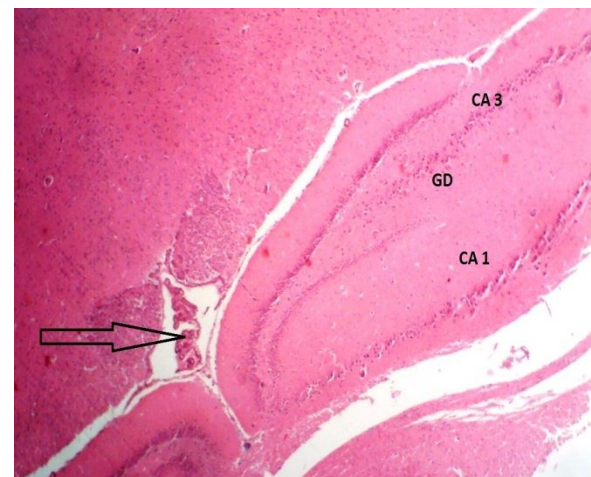
CONTROL (group 4) x 100 H&E

Photomicrograph shows section of the hippocampus showing distinct region of the hippocampus (CA1-CA3 and dentate gyrus GD). The cytoarchitecture of the neurons and glia cells (granular and pyramidal cells) appear essentially normal.



CONTROL (group 4) x400 H&E

Micrograph shows detail of the control animals, the nerve cells (NC), glia cell (GC) and neuropil (NP) appear normal.



GROUP 1 (2 WEEKS OF EXPOSURE) x100 H&E

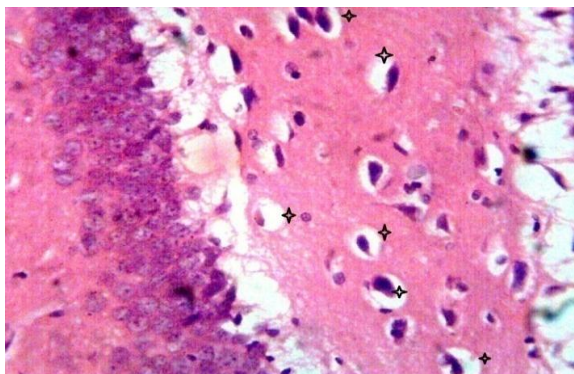
Photomicrograph shows section of the hippocampus showing distinct region of the hippocampus (CA1-CA3 and dentate gyrus GD). The cytoarchitecture of the neurons and glia cells (granular and pyramidal cells) appear essentially normal. Arrow shows the choroid plexus of the ventricle.



GROUP 2 (3 WEEKS OF EXPOSURE) x100 H&E

Micrograph shows foci of nerve degeneration (arrow) within the CA1 region of the hippocampus, the dentate gyrus (DG) and capillary (CAP) appear normal.

Microscopic examination of the hippocampus group 3.



GROUP 3 (4 WEEKS OF EXPOSURE) x400 H&E
Micrograph shows marked degenerative changes (star)(lost of nissl and vacuolation) in the neuron.

Table 1: Mean of body weight

Group	N	First Week	Second Week	Third Week	Fourth Week
1	3	206kg	215.7kg	223kg	232kg
2	3	216kg	224.7kg	231kg	237.7kg
3	3	179.3kg	194kg	202kg	209.3kg
4 (control)	3	154.3kg	158.3kg	162.7kg	167kg

Table 1 shows an increase in body weight of the rats in all groups from the first to the fourth group.

Table 2: Physical observation of rats in group 4 following exposure to paint fumes

	First Week	Second week	Third Week	Fourth Week
Food Intake	Decrease	Normal	Increase	Increase
Water	Decrease	Decrease	Increase	Increase
Defecation/Urination	Normal	Increase	Increase	Increase
Body Weight	Increase	Increase	Increase	Increase

Table 3: Physical observation of rats in group 2 following exposure to paint fumes

	First Week	Second Week	Third Week
Food Intake	Decrease	Normal	Increase
Water	Decrease	Decrease	Increase
Defecation/Urination	Normal	Increase	Increase
Body Weight	Increase	Increase	Increase

Table 4: Physical observation of rats in group 1 following exposure to paint fumes

	First Week	Second Week
Food Intake	Decrease	Normal
Water	Decrease	Decrease
Defecation/Urination	Normal	Increase
Body Weight	Increase	Increase

In the first week of exposure, there was a notable decrease in the intake of food and water in all the groups, while there was an increase in body weight, defecation and urination remained normal. In the second week, there was still a notable decrease in the intake of water but food intake was normal and there was an increase in the rate of defecation and urination. By the third week there was an increase in all parameters, this remained so even in the fourth week. This was done in comparison with the control group.

Table 5: Test with the open field apparatus

Group	Exploratory	Anxiety	Line Crossing
Control	High	Low	All lines
Group 1	Low	High	About 5-9 lines
Group 2	Lower	Higher	About 4-7 lines
Group 3	Lowest	Highest	About 0-5 lines

In the course of exposure it was noticed the rats undergoing exposure to paint fumes always stay together in colonies and hide in a corner. Following the exposure, they get very anxious and the more they are exposed, the more anxious they get.

DISCUSSION

The photomicrograph of the control group shows an essentially normal cytoarchitecture of the neurons and glia cells with distinct regions of the CA3 and CA1 regions and the dentate gyrus (GD). It was seen that there was no distinct change in the rats in group 1 (exposed for 2 weeks), while in group 2 (rats exposed for 3 weeks) there was nerve degeneration in the CA1 region although the dentate gyrus and capillary appear normal but in the last group: group 3 (rats exposed for 4 weeks), there was degenerative changes in the neuron and all through the cells of the hippocampus. In comparison with the control group it was noticed that a prolonged exposure to paint fumes will result to degeneration of the cells of the hippocampus. For the rats in group 2 as a result of the degeneration of the CA1 region, (the CA1 in a normal functioning hippocampus carries information from the CA3 to the subiculum and out of the hippocampus to the entorhinal cortex. It serves as an output channel of the hippocampus) there won't be

proper flow of information in the brain; this will lead to anterograde amnesia (inability of the brain to form new memories). Therefore the rats in group one will not be able to remember and recall old memories but may be able to form new memories, this is in line with the theories of Catherine (2006), Di Gennero (2006) and Squire and Schacter (2002) that states that a damage to the hippocampus will result in both anterograde and retrograde amnesia. The CA1 region also deals with spatial movement therefore rats here might not be able to remember and form new memories of a new environment they find themselves in. For the rats in group 1, there is a possibility of other occurring changes in the cells of the hippocampus which are not visible and cannot be seen with the H&E staining procedure. Therefore, one cannot ascertain the changes that occurred in the cells of the rats in group 1.

From the photomicrograph, it is clearly seen that there was marked degeneration of the cells of the hippocampus especially the Nissl bodies. These Nissl bodies act as endoplasmic reticulum in the brain as they synthesize proteins used in the transmission neurotransmitters. As a result of the degeneration of the Nissl bodies, neurotransmitters are not adequately synthesized. The mossy fibers of the cornu ammonis is said to be a neurotransmitter, as a result of the degeneration of the Nissl bodies, they will not receive information along the perforant pathway. This also will affect the dentate gyrus that functions as an encoding medium of the brain. Not only will there be no proper access to previous memories as a result of the degeneration of Nissl bodies, there won't be any formation of new memories. Peradventure new memories are formed as a result of knowing that there is also degeneration of the cells in the CA3 region, there will be no input of information in the hippocampus to anterograde and retrograde amnesia. The brain cells are the only cells in the body that do not regenerate when they degenerate. Therefore, as a result of the degeneration of these cells adverse effect such as amnesia will occur in the brain and these changes are not reversible.

As a result of the degeneration of the cells in the CA1 region, the spatial movement of the rats may have been disorientated and this can explain the study on the open field test. It was observed that the longer the time of exposure, the lesser their ability to cross the lines. The decrease in the locomotor and exploratory ability of the rats can be said to be as a result of the paint fumes when compared to the control group. Also noted was an increase in the rate of anxiety as exposure to paint fumes increases. According to Calm clinic (2015), anxiety can lead to many physical and mental health issues such as memory loss and weight gain can be an adverse sign of anxiety. This could be as a result of the cortisol level (cortisol is released in times of stress and in the course of exposure it was noted that the rats always search for fresh air in their exposing chamber and this can lead to stress), excess eating, or inactivity. Therefore it will not

be wise to say that the weight gain is as a result of the exposure to paint fumes (although that could be a reason) as there are other factors involved.

CONCLUSION

Paint fumes are hazardous to human health, as prolonged exposure to them will result in various health issues ranging from brain fog, leukemia, cancers and amnesia. As seen in this study, it was noted that prolonged exposure to paint fumes can induce anterograde and retrograde amnesia as a result of its effects on the hippocampus and behaviorally it can result in an increase in anxiety which could be as a result of an increase in the cortisol level and low exploratory level.

RECOMMENDATION

hippocampus stereology, Biochemical studies and special staining recommended for further studies to reveal hippocampal details.

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