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In vivo Evidence of Mice Hippocampal Alterations Resulting from High Dose Caffeine Exposure during Intrauterine Life

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AJO and SYO managed the analyses of the study. Authors JOO, AJO and SYO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Caffeine ingestion during pregnancy requires attention and investigation towards assessing the level of safety and possible effects on the brain and mental health. Caffeine ingestion during pregnancy has been discouraged despite concerns about lack of adequate facts to support this presumption and that such position is being taken based on speculations or inadequate facts. To this end, this research investigated the effects of caffeine exposure on hippocampus at various dosages during intrauterine life. Eighteen pregnant mice were divided into three groups A-C. Group A were fed *ad libitum* on mice feed pellets throughout the experimental duration to serve as control. Group B were administered the lower dosage [50 mg/kg body weight] of caffeine while Group C were administered high dosage [120 mg/kg body weights] of caffeine during intrauterine life by oral gavage. Brain tissues of the animals were excised after being sacrificed by cervical dislocation at

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Day 12 of postnatal life. The tissues were processed using the Haematoxylin and Eosin staining technique and the results were subject to histomorphological analysis. Caffeine at the high dosage substantially compromised hippocampal formation and dentate gyrus structural integrity; particularly by limiting cellular differentiation and elaboration of the cells. The differentiation of neurons into the typical pyramidal cells of the hippocampus was largely limited.

Keywords: Brain; hippocampus; neuron; pregnancy.

1. INTRODUCTION

The hippocampus is generally described as a part of the allocortex of the brain; its two major defining features include the Cornu Ammonis and the dentate gyrus [1]. It is understood that effects on this structure largely influences the process of memory consolidation, hence formation and storage. It is however important to note that this does not label this structure an archive but a memory processing centre. Major function of the hippocampus may be summarised to include the representation of self-location in cognitive dimensions as well as the storage and retrieval of memory [2]. As such, other implications of the hippocampal activities include learning, motivation and appetite control [3].

Caffeine is a popular neurostimulant consumed globally [4,5]. It has even been dubbed a legal drug. Interestingly, caffeine is found the world's major beverage drinks especially coffee, tea and cocoa. It is also a major additive to soda and energy drinks. Pure caffeine- whether in form of powder or pills- is often taken either for the purpose of *mental enhancements* in form of wakefulness and alertness or as a therapeutic agent. This wide range of uses makes various categories of people consume caffeine almost inadvertently on daily basis. Caffeine at normal dosage interacts with the adenosine receptors AR1 and AR2B but may also involve other adenosine receptors- AR2B and AR3 at higher dosages [4]. At doses that could be toxic to the body, it negatively influences homeostasis especially calcium metabolism and this produces deleterious effects. Caffeine effects, either on health in general or on the brain are influenced by dosage, age, sex and genetic factors among others.

Caffeine effects at moderate dosages have been described as beneficial to a large extent, though this remains continually debatable. Concerns are however raised when caffeine is consumed during pregnancy or the early stages of life when the brain is still largely vulnerable to the influence of caffeine. Interestingly, effects of caffeine exposure on the developing brain have been previously reported to include altered morphology, cognition and behaviour [6-9]. Whether such effects may possibly classified as being teratogenic remains debateable.

The aim of this investigation was to observe the effects of low and high caffeine doses exposure during the intrauterine life on the hippocampus; specifically by observing the histomorphological features of the hippocampus of the various animal groups.

2. MATERIALS AND METHODS

Pregnant mice, approximately 60 days old, [n=18] were grouped into three groups of six mice per group. Group A were fed *ad libitum* with standard mice pellets throughout pregnancy and served as control for other groups of animals. Anhydrous caffeine powder was dissolved in distilled water, and administered once daily. The second group [Group B] were administered a lower dosage of caffeine [50 mg/kg body weight] while the third group [Group C] were administered the higher dose of caffeine [120 mg/kg body weight] throughout the duration of pregnancy that lasted approximately 21 days. The route of administration was orogastric using suitable oral gavage. Groups B and C were also fed with standard mice pellets. The offspring were allowed to grow till the Day 12 of postnatal life [See Fig. 1]. They were thereafter sacrificed by cervical dislocation and their brain tissues were excised and processed using the haematoxylin and eosin staining technique. The results were represented by photomicrographs taken by the Accuscope Photomicrographic Set. The results were further subjected to qualitative histomorphological analysis with emphasis on general hippocampal architecture; cell spatial distribution and relative density and characteristic cell morphologies [10] among other considerations. All experimental procedures, especially animals handling and treatment were carried in compliance with the institutional ethical standards and requirements of the Ben Carson School of Medicine, Babcock University, Nigeria.

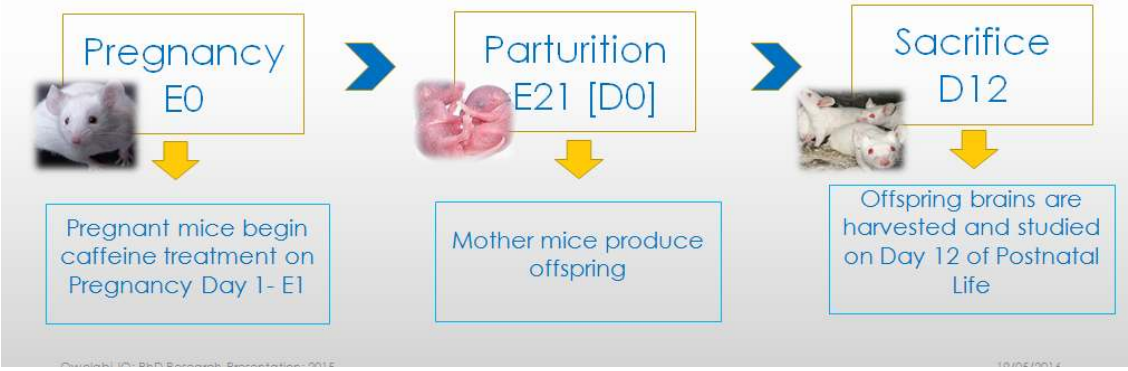

Fig. 1. Schematic illustration of the experimental design

3. RESULTS

Photomicrographs of the hippocampal formations of the experimental animals showed that there were structural aberrations that could be attributed to caffeine exposure.

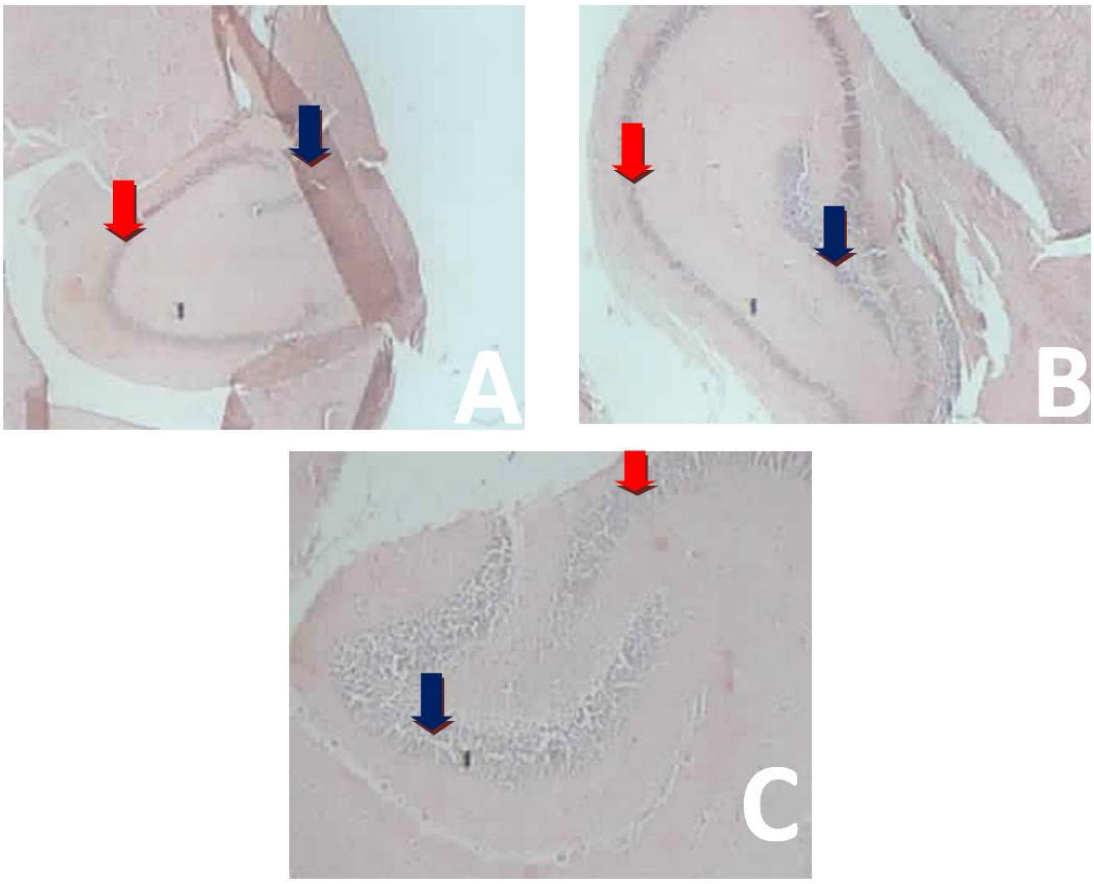

Fig. 2. Histological demonstration of the hippocampal formation showing the Cornu Ammonis and the dentate gyrus of the animal groups A, B and C as labelled. The hippocampal formation is roughly preserved as a whole, though evidences of alterations in the patterns of structural outline are observable [H&E, X160]

Legend: *Cornu Ammonis*
Dentate gyrus

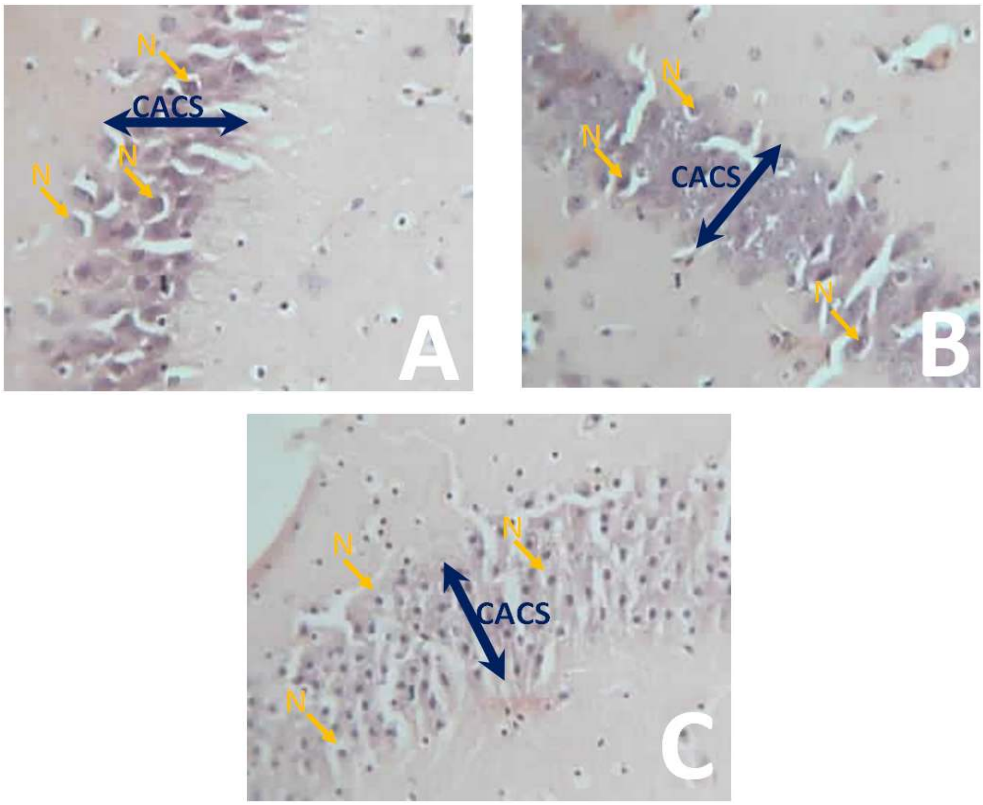

Fig. 3. Histological demonstration of the cornu ammonis across the experimental animal groups A, B and C as labelled. Caffeine exposure effects are observable in the pyramidal cells layer of the Cornu Ammonis; there are morphologic distortion of cells at the lower dosage and marked diminution of cells at the higher dosage. [H&E, X640]

Legend: N = Neuron; CACS = Cornu Ammonis Cross Section

4. DISCUSSION

It is interesting that the current investigation is one of the few to have considered the effect of caffeine intrauterine exposure on the hippocampal formation integrity. The investigation involved a qualitative ultrastructural analysis of the hippocampal formation- particularly the Cornu Ammonis and the dentate gyrus with emphasis on the predominantly cellular features. This implies that pyramidal cells of Cornu Ammonis and the granular cells of the dentate gyrus were primarily analysed. The consideration of the entire hippocampal formation, especially relative to its outline and general organisation provided insight into the interpretation of results and the understanding of the nature of effects. Caffeine generally affected the morphologies of the cells as well as their spatial differentiation and densities. There are aberrations in the hippocampal outline and such effect had been previously reported in adult hippocampal neurogenesis [5].

The general hippocampal formation outline is largely preserved in the Group B animals that were exposed to the lower caffeine dosage [Fig. 2B] and in the Group C animals that were exposed to excessive caffeine dosage [Fig. 2C]. There are however mild aberrations in terms of the outline, supposedly due to caffeine exposure [Fig. 2C]. The Cornu Ammonis is observable in all the groups as well as the dentate gyrus. This observation points to the fact that caffeine at any of the doses employed did not cause extensive disruption of the hippocampal formation, especially in a teratologic manner; but there are alterations in the outline of the Cornu Ammonis and the dentate gyrus. Whether these architectural aberrations are due to original alterations in the pattern of neuronal arrival and arrangement at this cortex or post-arrival neurogenesis may not be absolutely ascertained. Han et al. [5] however, had reported that adult neurogenesis was altered by caffeine exposure.

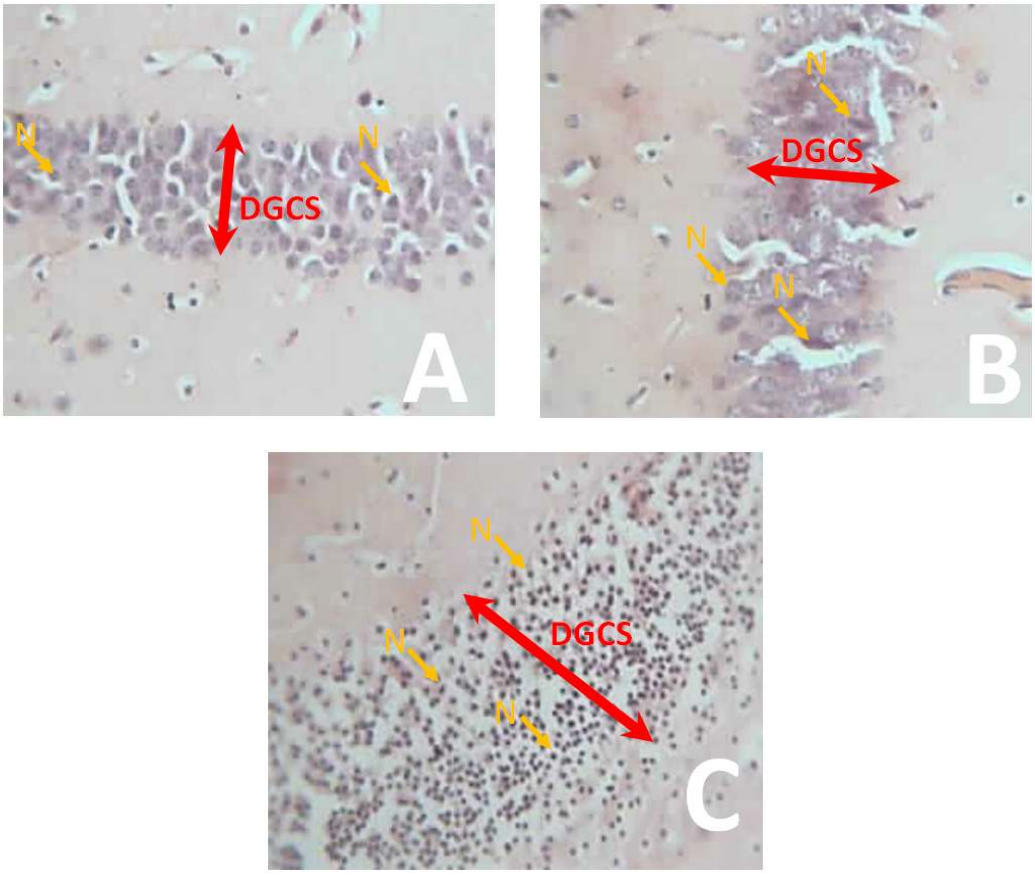

Fig. 4. Histological demonstration of the granular cells of dentate gyrus of the animal groups A, B and C as labelled. Photomicrographs show that caffeine exposure effects on the dentate gyrus include marked variations in cell population and density; excessive caffeine dosage exposure observably caused marked cellular diminution, but increased relative density [H&E, X640]

Legend: N= Neuron; DGCR = Dentate Gyrus Cross Section

The various parts of the Cornu Ammonis are demonstrated in the various groups [A, B and C]. Caffeine exposure altered neuronal morphology in dose dependent manner. Neurons appear heterogeneous in the Group C that was exposed to lower caffeine dosage and the cells are poorly defined. This observation suggests that caffeine affected the pattern of differentiation and elaboration of the pyramidal cells of the Cornu Ammonis [Fig. 3B]. However, it might not prevent the migration of the cells or reduce their number. Cells of the Group C animals that were exposed to the high caffeine dosage had altered morphology. They are relatively smaller in size and could barely be morphologically differentiated from the glia [Fig. 3C]. This observation suggests that the elaboration of these cells was retarded or limited in this group, consequent of caffeine exposure. Thus, a major effect of caffeine exposure during pregnancy included limitation in neuronal development and attainment of normal pyramidal morphology.

Caffeine also had effects on the granular cells of the dentate gyrus. Cells were poorly defined [morphologically] at the lower dosage of exposure. This implies that caffeine affected cell morphologies at the low dosage of exposure during pregnancy. At the higher dosage; caffeine seriously affected neuronal morphology and spatial distribution. Cells were relatively greater in density; however, they were smaller in size and appear poorly differentiated as neurons [Fig. 4C]. This observation again points to the fact that caffeine in this group limited the development and morphological differentiation of the cells. This effect altered the normal arrangement of cells and their typical types in the hippocampal

formation. This would most likely produce negative effects on hippocampal functions.

There is no doubt that the observed structural aberrations are associated with caffeine exposure. They would also most likely have observable effects on hippocampus functions such as cognition [11,12] and memory in various forms including the short term memory [STM]. Some existing literatures are in support of caffeine use as being beneficial while others reports negative effects [13-15]. Caffeine has been reported to affect long-term memory [LTM]: to have positive effects [16-18] and certain negative effects as well [19]. Notwithstanding, there are reports that suggested that caffeine did not influence long term memory [20,21]. Though the available literatures suggest variations in inferences and conclusions from the conducted investigations; what has to be understood include that dosage, duration of caffeine use, age of the subjects or objects and the design of experiment such as caffeine preparation and route of administration among other factors influence not just the results but also the perspective of the investigators' judgement. Therefore, the advantage of the current investigation is that it may provide information on the structural changes that occurred under caffeine influence, especially when exposure took place during development.

One of the closest contexts to the current investigation is the report of Han et al. [5]; stating that caffeine limits adult hippocampal neurogenesis. The contrast is that the current investigation would rather address pre- and postnatal neurogenesis. It is important to state that caffeine did not limit cellular proliferation. Hence, the population of the predominantly cellular layer and zones of the hippocampal formation in the current investigation is relatively not reduced. Rather, caffeine limits the elaboration of such cells, particularly morphologically. The hippocampal cells of the animals that were exposed to excessive caffeine dosage exposure were morphologically deformed. The underlying mechanisms would require continuous investigation. For instance, Souza et al. [22] reported that maternal caffeine exposure altered neuromotor development and hippocampus acetylcholinesterase activity in rats. Poole et al. [23] suggested that the developing hippocampus might be quite sensitive to caffeine influence or effects.

5. CONCLUSION

In conclusion, caffeine exposure during pregnancy and early postnatal life has effect on the hippocampal structural integrity. The effects include altered neuronal morphology and limitations in morphological differentiation and elaboration of individual cells. The pyramidal cells are typically affected. The general outline of the hippocampal formation is also slightly altered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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