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High-dose Caffeine Intrauterine Exposure Altered Neuronal Morphology and Spatial Distribution in Murine Models Cerebral Cortex

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Authors' contributions

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

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ABSTRACT

Caffeine is a neurostimulant that is globally consumed with little or no restrictions by various groups of people. Though concerns have been raised over the possible effects of caffeine exposure during intrauterine life or pregnancy; there are not enough empirical evidences to establish the specific potential effects of caffeine exposure on the devolvement and the functions of the brain. The current investigation examined the possible effects of caffeine exposure at various dosages modelled after human consumption quantities in murine models. The experimental animals-pregnant mice, were grouped into four and labelled A, B, C and D respectively. Anhydrous caffeine was dissolved in distilled water and administered to animals daily using oral gavages. The Control Group was labelled A and the pregnant mice were fed *ad libitum* throughout the experiment to serve as the reference and normal in the context of results interpretation; the Group B animals were administered the moderate caffeine dosage [10 mg/kg body weight]; Group C received moderately high dosage [50 mg/kg body weight] and the Group D received the excessively high caffeine

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dosage [120 mg/kg body weight] throughout pregnancy- from the day 1 of pregnancy to parturition. The mice litters were allowed to develop until Day 12 Postnatal life, and then sacrificed. The brain tissues were excised and processed using the Haematoxylin and Eosin histological technique. Histomorphological results showed that caffeine exposure at the excessively high dosage altered neuronal morphology and spatial distribution with evidence of limitations in dendritic and axonal development and elaboration. Caffeine at the lower dosages showed potentials to mildly influence neuronal spatial distribution; with glias not showing extensive morphological or spatial alterations.

Keywords: Caffeine brain; pregnancy neuron glia.

1. INTRODUCTION

Caffeine is taken in various forms and quantities and it is the most popularly consumed neurostimulant globally [1-3]. It is present in certain plants which are consumed as food and drinks and these include coffee [seed]- which is bred and taken as a beverage drink; tea [leaf] also typically brewed and taken as a beverage drink; cocoa [bean]- usually consumed in various preparations including beverage drink, chocolate and candies among others [4,5].

Apart from the consumption of caffeine from its natural sources, it also taken as an additive to medicinal drugs and can be used on its own for certain medicinal purposes [6]. Also, caffeine- as a pure extract is often taken in form of powder [usually dissolved in water or drinks] and in forms of tablets. Other means of consuming caffeine include consumption in soft drinks or soda as well as a number of energy drinks [6]. Hence, caffeine is consumed by a great number of people globally on a daily basis and almost unavoidably.

Interestingly, pregnant women are also not excluded for the caffeine use and often, its abuse. There have been argument and counterarguments concerning the safety of caffeine use in pregnancy; especially with respect to the developing human whose brain is expected to the susceptible to the influence of such agent. However, there is the obvious need for more empirical reports on the specific effects of caffeine on the developing brain as well as the possible benefits or consequences of such- if there are.

In mammals generally, the process of brain development include series of stages that involve the original or primordial neural tube first developing into three regions named the fore, mid and hindbrain in cranio-caudal orientation respectively [7]. The cerebrum develops as the anteriormost part of the fore brain, as known as the telencephalon; and it is often referred to as the higher centre [7]. Its prefrontal cortex is associated with executive functions that particularly premise factors of personality, behaviour, emotion, intelligence and other related attributes [8]. Aberrations of these attributes are also closely related to abnormalities of the frontal lobe or its cortex. Caffeine primarily interacts with adenosine receptors [AR]; it would interact conventionally with the AR1 and AR2A at normal dosages, but with AR2B and AR3 at excessively high dosages [2].

A number of investigations have suggested that caffeine reportedly affected brain development by altering neural tube closure [9]; also by causing reduction of glial fibrillary acidic protein [10]. It has also been reported that caffeine exposure affected the pattern of neuronal development [11]. More so, effects including the acceleration of telencephalic vesicle evagination in mouse embryos [12]. It has been suggested that some of caffeine effects on neurocortical features might have resulted from caffeine's inhibitory action on the contractile activity of apical microfilament bundles in developing neuroepithelial cells [13].

2. MATERIALS AND METHODS

Pregnant mice were exposed to various regimens of caffeine throughout pregnancy. The animals were divided into four groups designated A-E. Group A served as the control groups while Groups B, C and D served as the caffeine-exposed groups to evaluate the effects of various dosages of caffeine on the developing brains into the animal groups during intrauterine life. It is being estimated that 10 mg/kg/day is equivalent to 2–3 cups of coffee per day in humans based on a metabolic body weight conversion [14]. The patterns of grouping and the rationales are further described as below:

Group A: Control murine models that were only fed *ad libitum*; the rationale is to have a

group of animals under normal and uninfluenced developmental condition, hence that could serve as suitable and contextually reliable reference for the caffeine-treated groups.

Group B: Murine models exposed to low-dose caffeine during intrauterine life. Pregnant mother-mice were given 10 mg/kg body weight of caffeine daily. This represented habitual moderate caffeine consumption in humans.

Group C: Murine models exposed to medium-dose caffeine during intrauterine life. Pregnant mother-mice were given 50 mg/kg body weight of caffeine daily. This represented high caffeine consumption in humans.

Group D: Murine models exposed to high-dose caffeine during intrauterine life. Pregnant mother-mice were given 120 mg/kg body weight of caffeine daily. This represented excessive and abnormal caffeine consumption in humans.

The treatments as described lasted throughout the time of pregnancy. The neonates were left to develop till the 12th day of postnatal life. They were sacrificed and the brains were excised, fixed in formal saline and processed using the routine eosin and haematoxylin staining technique [15]. Photomicrographs of the brain sections were taken using the Accuscope® Photomicrographic Set. The results were presented as photomicrographs. Histomorphological reports were given with particular interest on neuronal and glial morphology, spatial distribution and density [16] in each caffeine-treated animal group while the Control Group A served as the reference. The research and methodology was approved by the Standard and Ethics Committee of the Ben Carson School of Medicine, Babcock University, Nigeria.

3. RESULTS AND DISCUSSION

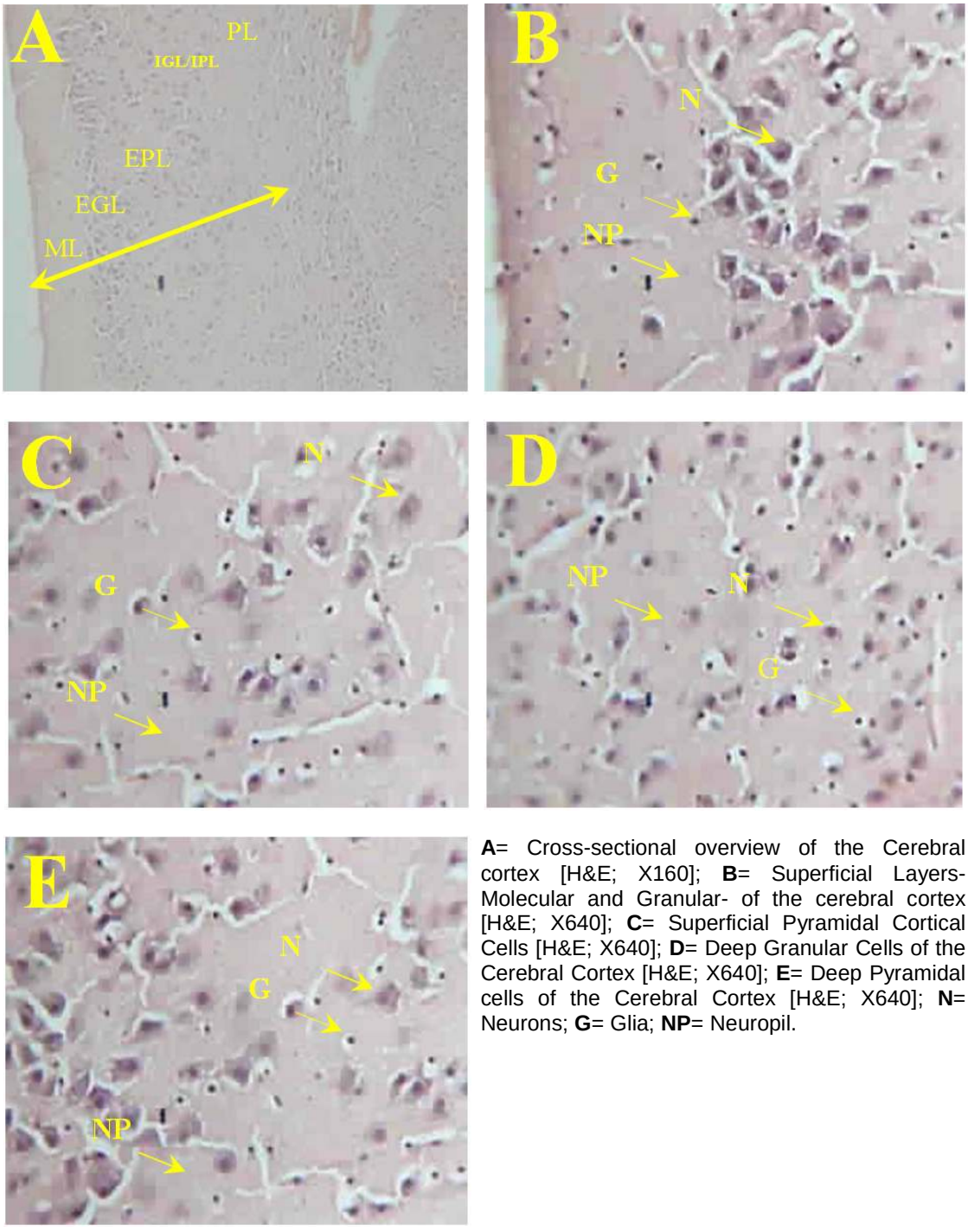
The Control Group A served as the standard reference group. Neurons and glia were clearly demonstrated across the cortical layers of the mice brains. The various layers of the cortex were clearly demonstrated- the outermost molecular and the underlying pyramidal and granular layers [17]. The cells in these layers are demonstrated and as such the control cortex serves as a suitable reference for the other photomicrographs of the treated animal groups.

Caffeine influenced neuronal morphology at the lower dosage [10 mg/kg body weight] of administration [Group B; Fig. 2]. The cortex is normally demonstrated to a large extent. Pyramidal cells are relatively prominent; also, granular cells are relatively abundant. However, cells within the deeper granular layer are relatively closely packed with certain cells appearing heterogeneous both in terms of staining intensity as well as sizes. Caffeine thus included the distribution of cells as well as their terminal morphology. It is important to note however, that they are not enough aberrations to suggest obvious negative consequences. This nature of caffeine effect had been previously suspected to be positive [18].

The spatial distribution of cells was affected when animals were administered the high dosage [50 mg/kg body weight] of caffeine [Group C; Fig. 3]. The cortex of the experimental animals is largely preserved; however, granular cells in particular are relatively prominently demonstrated. A number of them are also heterogeneous in morphology. Thus, neuronal spatial distribution is affected in this group and neuronal morphology is also influenced by caffeine at the particular dosage.

Neuronal differentiation is substantially affected as well as morphology in the Group D [Fig. 4] mice brains that were exposed to excessively high dosage [120 mg/kg body weight] of caffeine. The cortical layers were poorly defined relative to the Control Group A. Neurons were particularly smaller in size and they exhibit morphological aberrations. Granular cells barely differ from the glia. Their small size suggested limited elaboration not just, morphologically but also in terms of axonal and dendritic structures. Pyramidal cells are also poorly differentiated and defined in the cortical tissue. They are smaller, individually and less prominently demonstrated. The deeper granular cells are quite poorly demonstrated and show not much difference from the glia in terms of morphological differentiation and elaboration. This might not be unconnected with the variations in the mechanisms of caffeine-AR interactions at excessively high dosages relative to the lower and conventional dosages [1,2].

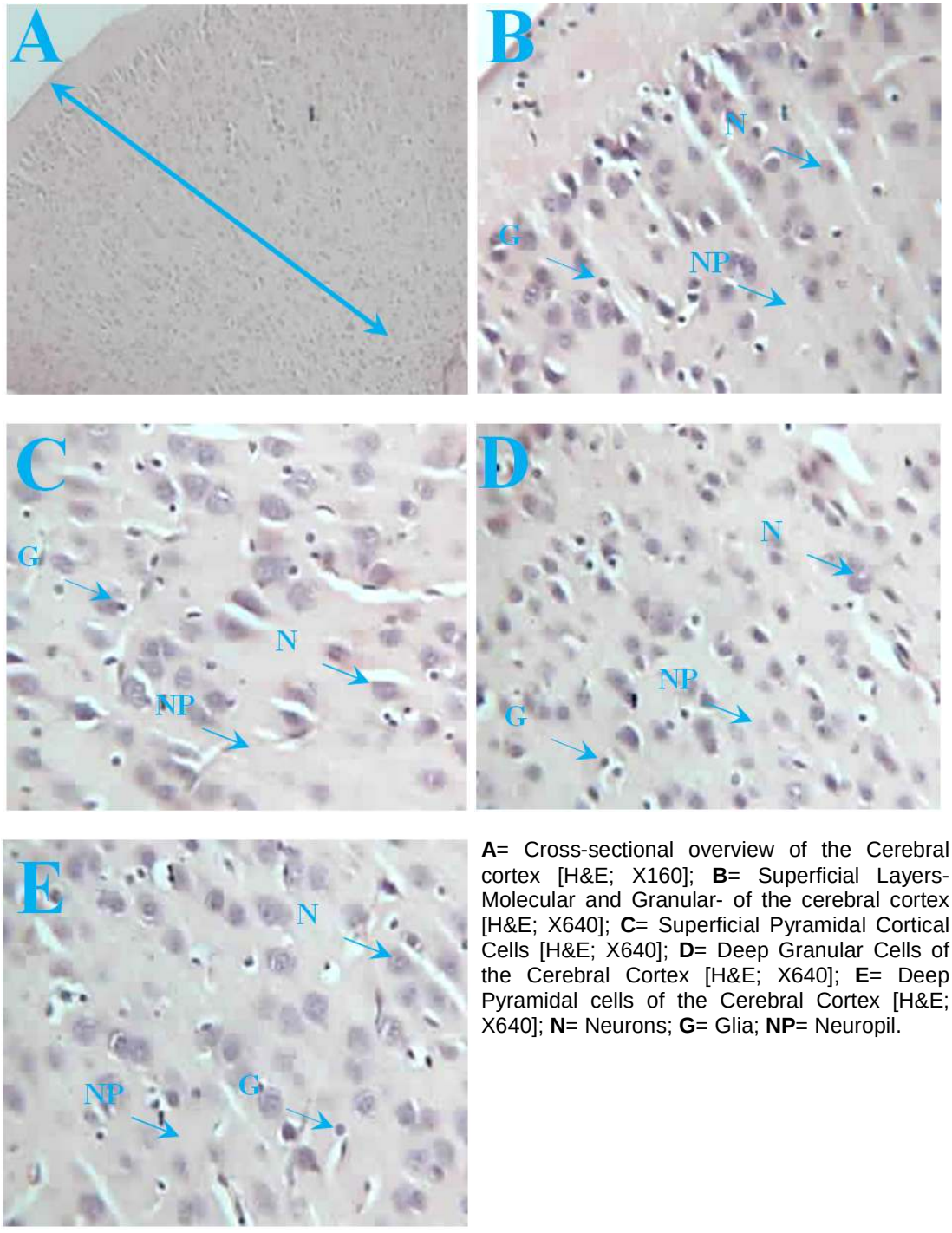
Group A



A= Cross-sectional overview of the Cerebral cortex [H&E; X160]; **B**= Superficial Layers- Molecular and Granular- of the cerebral cortex [H&E; X640]; **C**= Superficial Pyramidal Cortical Cells [H&E; X640]; **D**= Deep Granular Cells of the Cerebral Cortex [H&E; X640]; **E**= Deep Pyramidal cells of the Cerebral Cortex [H&E; X640]; **N**= Neurons; **G**= Glia; **NP**= Neuropil.

Fig. 1. Photomicrographs of the frontal cortex of mice which mothers were fed *ad libitum* during the intrauterine life to serve as control. Neurons and glia are well demonstrated across the cortical layers

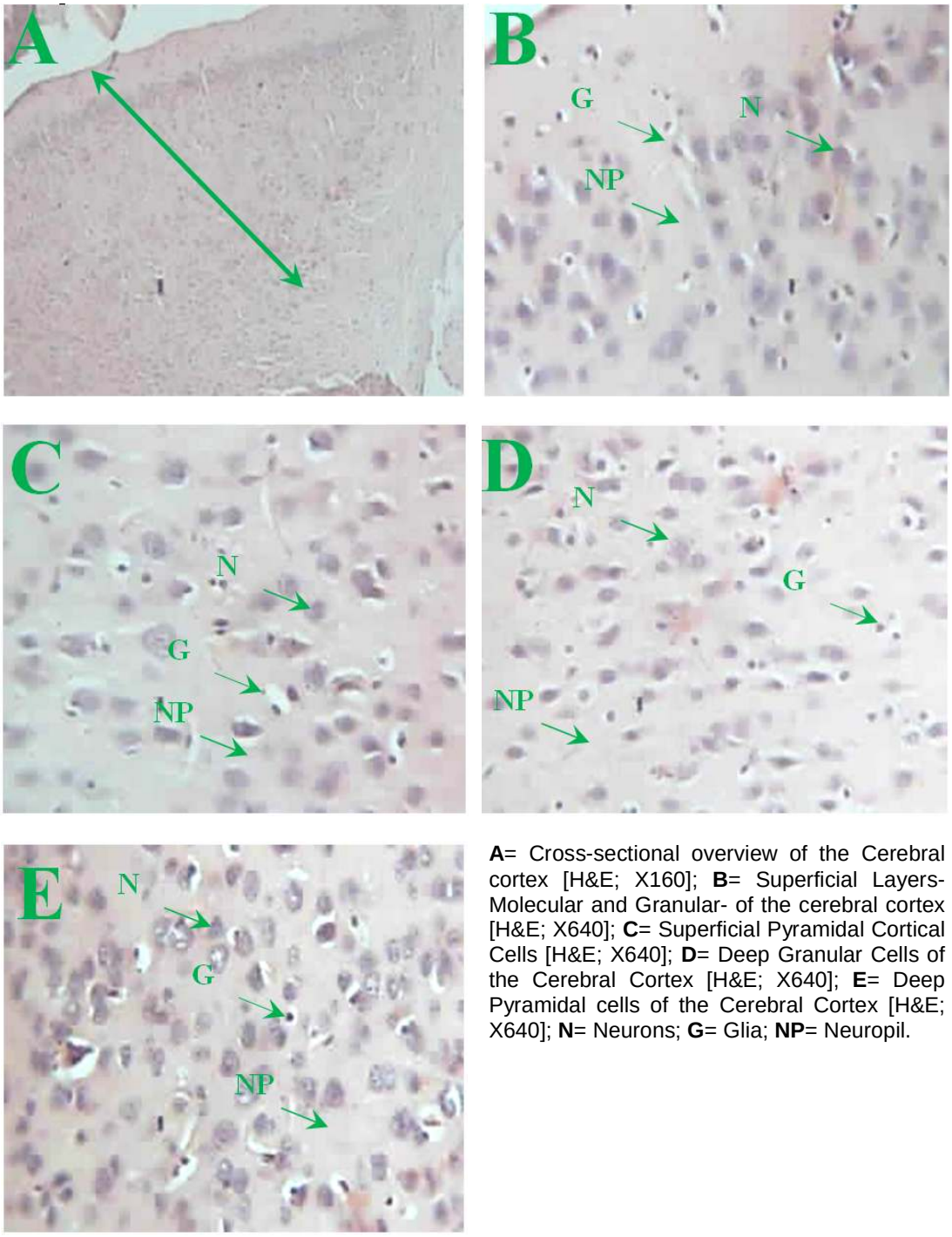
Group B



A= Cross-sectional overview of the Cerebral cortex [H&E; X160]; **B**= Superficial Layers- Molecular and Granular- of the cerebral cortex [H&E; X640]; **C**= Superficial Pyramidal Cortical Cells [H&E; X640]; **D**= Deep Granular Cells of the Cerebral Cortex [H&E; X640]; **E**= Deep Pyramidal cells of the Cerebral Cortex [H&E; X640]; **N**= Neurons; **G**= Glia; **NP**= Neuropil.

Fig. 2. Photomicrographs of the frontal cortex of mice which mothers were fed exposed to low dosage of caffeine during the intrauterine life. Neurons and glia are well demonstrated across the cortical layers; neurons are relatively prominently demonstrated [H&E X640; X1600; N= Neuron; G= Glia]

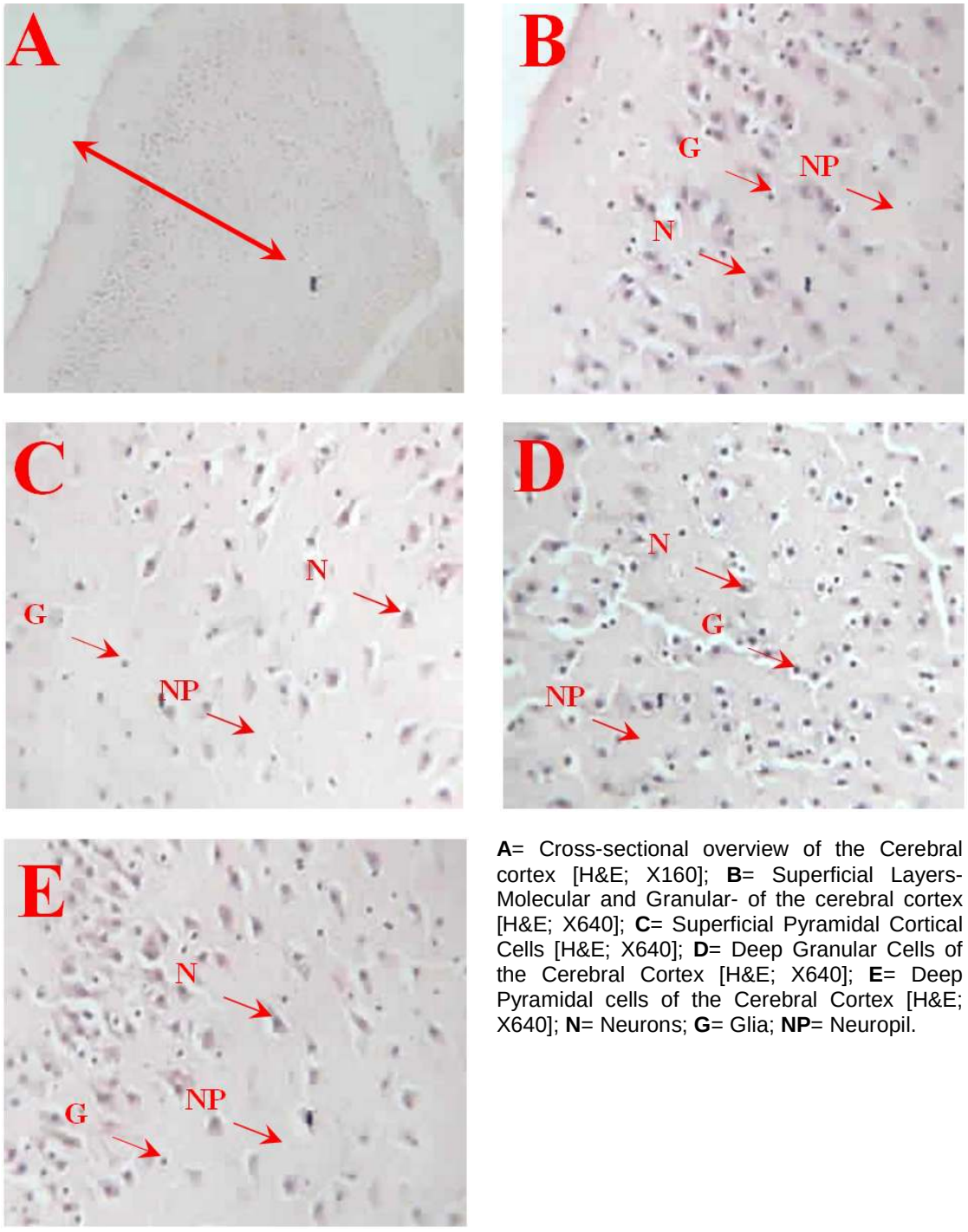
Group C



A= Cross-sectional overview of the Cerebral cortex [H&E; X160]; **B**= Superficial Layers-Molecular and Granular- of the cerebral cortex [H&E; X640]; **C**= Superficial Pyramidal Cortical Cells [H&E; X640]; **D**= Deep Granular Cells of the Cerebral Cortex [H&E; X640]; **E**= Deep Pyramidal cells of the Cerebral Cortex [H&E; X640]; **N**= Neurons; **G**= Glia; **NP**= Neuropil.

Fig. 3. Photomicrographs of the frontal cortex of mice exposed to high caffeine dosage through the mother during the intrauterine life. Neuronal spatial distribution is affected; neuronal morphology is also heterogeneous

Group D



A= Cross-sectional overview of the Cerebral cortex [H&E; X160]; **B**= Superficial Layers-Molecular and Granular- of the cerebral cortex [H&E; X640]; **C**= Superficial Pyramidal Cortical Cells [H&E; X640]; **D**= Deep Granular Cells of the Cerebral Cortex [H&E; X640]; **E**= Deep Pyramidal cells of the Cerebral Cortex [H&E; X640]; **N**= Neurons; **G**= Glia; **NP**= Neuropil.

Fig. 4. Photomicrographs of the frontal cortex of mice exposed to excessively high caffeine dosage through the mother during the intrauterine life. Neuronal differentiation is substantially affected as well as morphology and many cells appear morphologically distorted

These observations generally show that caffeine limited neuronal elaboration and differentiation in this group of animals; and different cells are affected- both granular and pyramidal. Histomorphological evidence from the current investigation show that caffeine interacted with the developing brain to produce sustained or persistent effects on the cells morphologies and spatial distribution [18]. However, the effects vary significantly based on the dosages being employed. At the low or moderate dosage being employed; there are mild evidences of cellular elaboration and relative prominence. A report on caffeine postnatal exposure suggested that moderate caffeine use influenced pyramidal cells by causing extensive dendritic differentiation and length [18]. The current evidence however shows that caffeine at excessively high dosage- as used in the Group D- could be deleterious. This might be due to the fact that caffeine at such high dosage would not just interact conventionally with the AR1 and AR2A; but would also recruit AR2B and AR3 while also altering calcium metabolism cum homeostasis [1,2,19-24]. It was reported to also cause cortical network reorganisation [25]. It may not be determined from the photomicrographs if these effects would produce negative effects or consequence on the other hand. Caffeine affected the morphologies of cells to a greater extent at the high dosage being employed and this study suggests that caffeine exposure at such a dosage might alter neuronal morphology and spatial distribution, possibly permanently. The effects based as the excessive dosage being used clearly show that caffeine exposure at this dosage might permanently and substantially limit neuronal differentiation as well their elaboration. Though there is not enough evidence to suggest cell deaths caused by the substance and the cells density would not suggest that; the cells are not normal morphologically and this would obviously limit their functions as well. These findings no doubt still support many existing literatures that have raised concerns about the safety of unregulated caffeine use relative to its effects on brain health during development [26,27] or postnatal stage of life [28,29].

It is therefore simply logical to infer that caffeine at such excessively high dosage would limit cortical cells development. Again, this means that cells would originate from the primordial neural tube and migrate to their destinations but might not attain their normal matured level of differentiation and elaboration. Since the cells typically appear not quite different from the glia, especially the astrocytes; it may require further investigations to validate the exact mechanism- whether the initially differentiated neurons did not reach their ultimate forms or the differentiation of the primordial cells into neurons and glia was being compromised, noting that the animals were exposed to caffeine throughout the entire duration of pregnancy.

4. CONCLUSION

It is therefore logical to conclude that caffeine affected the morphologies of the neurons in manners that correlate with the dosages being employed and excessive dosage of caffeine during pregnancy would greatly limit neuronal differentiation and elaboration. This would expectedly have significant functional consequences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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