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Neurodegeneration in Superior Colliculus of Neonatal Rats Exposed to Ethanol *In Utero*

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ABSTRACT: Alcohol has been established as a leading cause of developmental errors and, in man, alcohol consumption can interfere with vision. This study was carried out, therefore, to determine whether prenatal ethanol exposure can affect the histoarchitecture of the intracranial visual relay centres in neonatal rats. Twenty pregnant rats were randomised into two groups. Animals in Group A were allowed liberal access to 20% ethanol in 2% sucrose solution throughout their gestation period while Group B rats received distilled water instead. Litters were scarified on days 0, 4, and 7 post parturition and the superior colliculi were recovered, fixed in 10% formol calcium, and processed for routine H&E and Nissl stains. Photomicrographs obtained revealed histological alterations in the superior colliculi and in the staining intensity of the nuclear materials and Nissl bodies. This study provides evidence that suggest that prenatal ethanol exposure causes neurodegeration in the visual relay centres of neonatal rat.

Keywords: Ethanol, Prenatal, Neurodegeration, Superior colliculus,

Introduction

The effects of alcohol on mammalian development is intensively investigated and is beyond disputation that alcohol is a teratogen in many species, and particularly in man. Evidence that prenatal ethanol exposure (PEE) may cause error in the migration of neurone in the developing brain was first presented by Claren *et al* (1978) when they performed an autopsy on four brains of children diagnosed with fetal alcohol syndrome (FAS); in which ectopic neurones were observed in the white matter. Couller *et al* (1993) reported absent olfactory bulbs and tracks, poorly developed optic track, fewer cells in dentate gyrus and cerebellum the autopsied brain of a two month infant whose mother reportedly binge drank alcohol during her first trimester.

It can be argued that all the cellular effects of PPE can not be obtained in human autopsies because only few of such autopsies are performed. This is one reasons that makes animal studies very critical to our understanding of the effects and mechanisms of PPE on mammalian development. Animal studies have revealed microencephalic effects of PPE (Gilbert 2000), significant reduction in weight of forebrain, brain stem, and cerebellum (Maier *et al* 1997). Goodlett *et al* 1990 showed that, in rat, one day ethanolic exposure as high as 6.6g/Kg body weight (BW) is capable of inducing growth deficit in specific regions of the brain such as the cerebellum. Neurodegeration has been suggested as the process leading to ethanol-induced reduction in brain weight and volume. (Olney *et al* 2002).

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Although selective programmed neural death is a normal aspect of central nervous system (CNS) development; excessive neuronal death disrupts development of normal neural networks and may lead to cognitive behavioural dysfunctions (Chen *et al* 1998). There is evidence that specific regions and cell types are affected by PEE (Livy *et al* 2003). Rat models of PEE have clearly demonstrated that Purkinje cells, pyramidal cells in parts of Hippocampus are especially vulnerable to alcohol-induced neural loss (Chen 1998; Livy 2003). Livy *et al* 2001 provided evidence that neurons in ventrolateral nucleus of the thalamus and locus coeruleus do jot capitulate to ethanol-induced neurodegeration.

Previous investigations have shown that PEE negatively affect dendritic atructure and spines of neurons in the substantia nigra, the cortex, and hippocanpus (Tereho-Acuma *et al* 2000; Yanni and Levy *et al* 2000). Such dentritic anomalies can compromise the accuracy and fidelity of neural communication since dendrites are sites of synaptic connections for neurones (Macer *et al* 1997). In fact, the work of Yanni and Lindsley (2000) suggest that alterations in dendritic morphology and or decrease in dendritic spines interfere with optimal neural impulses.

Vision is very central to the survival of nearly all mammalian species and rat is not an exception. Rat like man has two intracranial visual relay centres but for its shorter gestation period, the visual relay of rat begins to develop on day 12 of gestation unlike in man where development begins on.

However, it is apparent that the effects of prenatal ethanol exposure are yet to be completely documented, as there was no literature, to the best of our knowledge, reporting the effect of PEE on neonatal visual relay centres. We therefore, attempted to answer the question 'does PEE affect the micromorphology of the neurones in the LGB and SC of neonatal rats?'

Materials and Methods

Ten rats (2 males and 8 females) from the same parent weighing 205±10g were procured from the department of Pharmacy Obafemi Awolowo University (OAU) and housed in individual cages under natural light and dark cycles at room temperature in the Animal Holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile Ife, Nigeria. Animals were allowed access to standard rat pellet and water *ad libitum.* The estrous cycle of the female rats were determined using Long and Evans (1922) modified by Mandl, (1951) and further modified by Marcondes *et al* (2002) method, before the mating with the viable males by using the "hand breeding" method in which the female was only introduced to a male on the day she is in "heat".

The pair then spent several hours together in a single cage for sixteen hours (4.00p.m. to 8.00a.m.) Vaginal smear using Marcondes, *et al* (2002) method, was carried out to confirm that mating has taken place and this was taken as day zero of pregnancy (Fakoya and Caxton-Martins 2006). Placing a male rat with a female when the latter is confirmed to be in heat, the animals were bred to the filial generation. Litters were always separated from dam when they are four weeks old. Twenty female rats from the third filial generation weighing 200±6g (aged ten weeks) were randomized into two groups A and B (n=10/group) and were allowed to become pregnant as described above. These rats were fed, *ad libitum*, with rat pellets purchased from Ladokun Feeds Ltd, Ibadan, Nigeria.

Rats were weighed daily to determine the progressive weight changes in each group during their gestation period. The rats in group A were given 20% ethanol in 2% sucrose solution *ad libitum* throughout their gestation period. While animals in group B, the control, were allowed access to distilled water *ad libitum* throughout their gestation period. The fluids were each replaced with fresh every twelve hours (at 0700 and 1900 GMT). The fluid intake was measured daily for each group to determine the progressive average fluid consumption through their gestation period. In order to prevent others from crushing the litter, the expecting doe was placed into a separate cage five days before parturition. This also gave her time to become acquainted with her new surroundings. The litters were weighed as soon as they were delivered. On days 0, 4, and 7, eight litters from each group were killed by rapid decapitation, their skulls open up with a born crusher and brains recovered, examined for any physical deformity, dried on filter papers. The superior colliculi and lateral geniculate bodies were dissected out and fixed in 10% formol calcium for 24 hours. Fixed tissues were latter embedded in paraffin wax and processed for histological studies. Serial sections of 5µ were either stained with hematoxylin and eosin as described by Drury and Wellington (1980) for histoarchitectural studies or Cresyl violets (Vogt 1932) for histochemical demonstration of Nissl bodies. Slides were viewed with under an Olympus binocular microscope interfaced with a JVC 3-CCD color video camera connected to a computer. The micrograph displayed on the screen of a desktop was captured, examined, and labeled.

Results

Micrographs obtained from sections that were stained with H&E revealed differences in the histology of animals exposed to ethanol utero compared to the control rats. Partial neural degenerations were observed in treated group from birth till day 7 of neonatal life. Plate 1a reveals normal microanatomical appearance of SC of rat at birth. Plate 1b, which was obtained from 0 day old rat that was prenatally exposed to ethanol reveals swollen nuclei and vacuolations suggesting neural degenerations. In Plate 2a and 2b, difference persist in the intensity of nuclear staining and vacuolations which are observable features of rat neonate exposed to ethanol utero. In Plate 3a and 3b differences in vacuolation (more in 3b) are less.

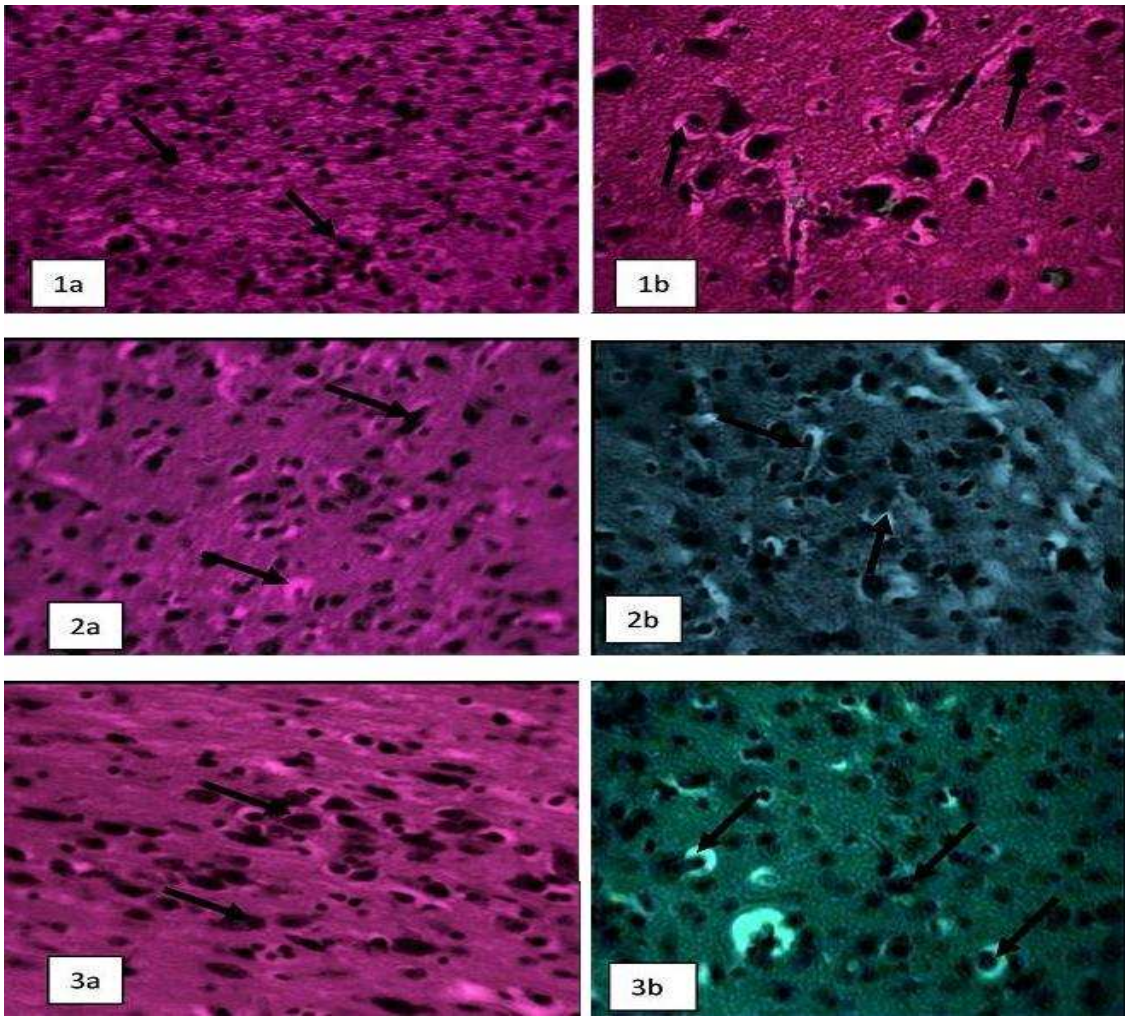

Plate 1a is a photomicrograph of a cross section of the Superior colliculus of 0 day old rat administered with distilled water. The nuclei (indicated with arrows) of neurons are normal. X400 (H&E)

Plate 1b is a photomicrograph of a cross section SC of 0 day rat prenatally exposed to ethanol; the nuclei appear swollen and vacuolations can be seen. X1200 (H&E)

Plate 2a is a photomicrograph of a cross section of the Superior colliculus of 4days old rat administered with distilled water. The nuclei (indicated with arrows) of neurons are normal. X1200 (H&E)

Plate 2b is photomicrograph of a cross section of the Superior colliculus of a 4day old rats that was prenatally exposed to ethanol; note vacuolations and swollen nuclei. X1200 (H&E)

Plate 3a is a photomicrograph of a cross section of the Superior colliculus of a 7day old control rat administered with distilled water. Neurons appear normal and vacuolations are absent. X1200 (H&E)

Plate 3b is a photomicrograph of a cross section of the Superior colliculus of a 7day old prenatally exposed to ethanol. Vacuolations are still visible. X1200 (H&E)

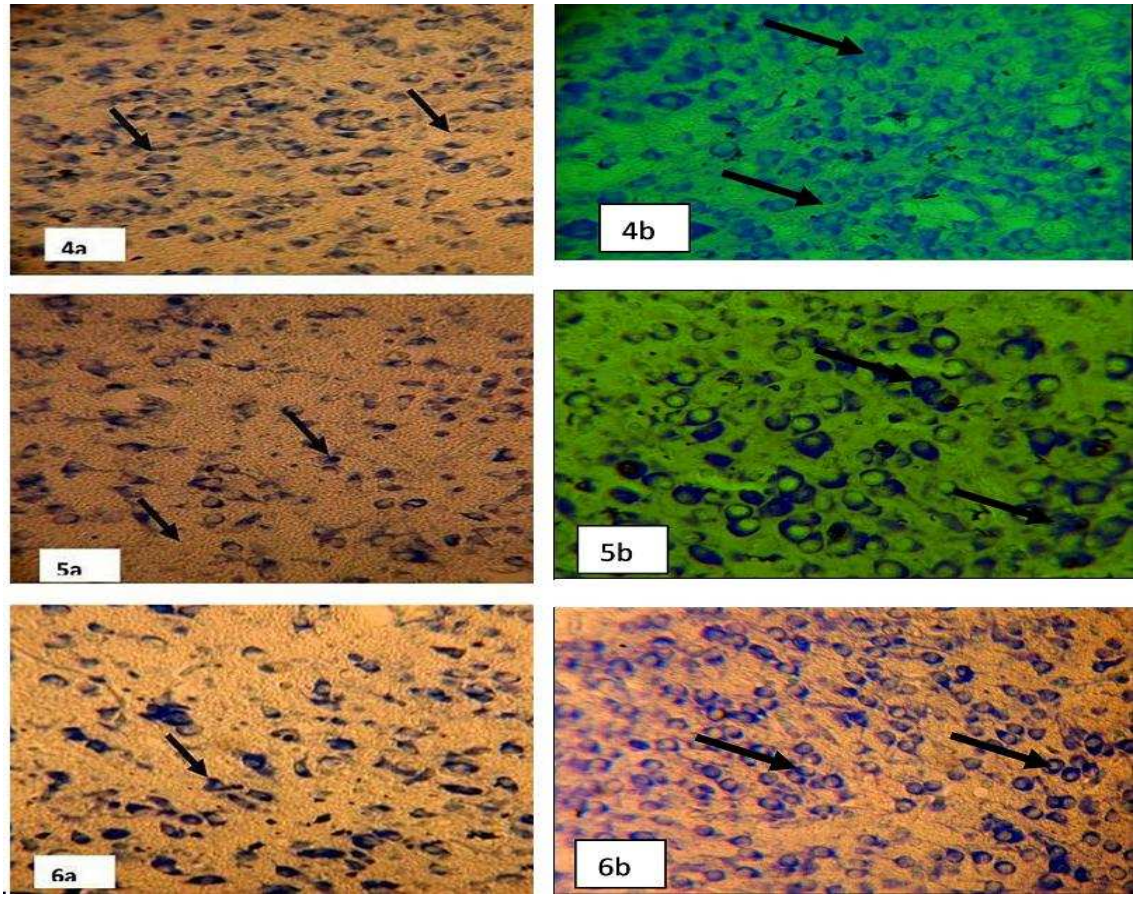

Plate 4a is a photomicrograph of a cross section of the Superior colliculus of 0day old rat administered with distilled water. The Nissl substance (indicated with arrows) of neurons stain normally. X800 (Nissl stain)

Plate 4b is a photomicrograph of a cross section SC of 0 day rat prenatally exposed to ethanol; the nuclei stain more intensely. X800 (Nissl stain)

Plate 5a is a photomicrograph of a cross section of the Superior colliculus of 4days old rat administered with distilled water. Nissl body stain normally. X800 (Nissl stain)

Plate 5b is photomicrograph of a cross section of the Superior colliculus of a 4day old rat that was prenatally exposed to ethanol; Nissl bodies are heavily stained. X800 (Nissl stain)

Plate 6a is a photomicrograph of a cross section of the Superior colliculus of a 7day old control rat administered with distilled water. Nissl stain is normal X800 (Nissl stain)

Plate 6b is a photomicrograph of a cross section of the Superior colliculus of a 7day old prenatally exposed to ethanol. Intensity of Nissl staining is comparative more. X800 (Nissl stain)

Discussion

The paucity of data of the correlation of PEE and morphogenesis of the intracranial relay centres prompted this investigation. It is now widely known alcohol is one of the few substances that cross both the brain and placenta barriers hence, it much documented deleterious effects of the developing CNS. The results of this study reveal that differences exist between the histology of superior colliculus of neonatal rats that were prenatally exposed to ethanol. Plates 1-3 compares the histological appearances of experimental neonatal rats and control at days 0, 4 and 7 post parturition and it is observable that neonate that were exposed to ethanol *in utero* exhibit abnormal histology in their superior colliculi. Specifically, in ethanol exposed rats, the microanatomy of the superior colliculi seem to

have suffered some dysgenesis as apparent from their swollen nuclei and many vacuolations. Although, the degree of vacuolation and nuclei swollen appear to abated on day 7, it is far from been totally eliminated which means the effect PEE on SC persist up to day 7 of neonatal life.

Histochemical demonstration of Nissl bodies (Plates 4-6) reveal more intensity of staining in the SC of rats prenatally exposed to ethanol (compared to those of control) at day 0, 4, and day of neonatal life. The more intensely stained Nissl bodies in the cell bodies and dendrites the SC of treated rat indicate increase proteins, cholesterol and neurosecretory proteins synthesis. A more pronounced increased in the synthetic activities of nervous tissue often result when the neuron are attempting to fight the degenerative effects of an assault. Neurodegerative effects of PEE similar to those observed in this investigation have been reported for other regions of the brain. (Coulter 1993) Ethanolic assault on developing brain can trigger excessive neuro-appoptosis because of the resulting imbalance in reactive oxygen species (ROS) generation and antioxidant defence system of the developing brain. ROS generation, with the pernicious effects of reduced capacity for neural glucose uptake and lipid peroxidation of neuromalema, is reportedly elevated in the brain of alcoholics (Ortiz *et al* 1995), which may lead to compensative protein synthesis evident in intense Nissl staining. The synergy of these negative effects of ROS may explain excessive neural loss found in the autopsied brain of FAS and possibly the one observed in this study.

The short-term effect of alcohol on vision in man is well documented but it not known whether alcohol-induced bad vision is connected to neural loss or degeneration in any of the visual relay centres. However, this study points to the fact that PEE may affect the developmental biology of the intracranial visual relay centres and cause early and abnormal neural death in the neonate. Whether this neonatal neural loss persists into adulthood remain to be investigated.

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