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The Effect of Glucocorticoid (Dexamethasone) Administration on the Postnatal Development of the Hippocampus and Growth of the Wistar Rat

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ABSTRACT

Synthetic Glucocorticoids are commonly administered to early low birth weight infants to prevent the onset of chronic lung disease. During this period, the brain is undergoing significant structural and functional changes and is vulnerable to external influences. This study observed the effect of early administration of glucocorticoids on the developing hippocampus. Wistar rat pups were grouped into ten groups of six pups each. Five of the groups made up the control, the remaining five were the treatment groups. 0.5mg/kg of dexamethasone was administered to four day old pups for a period of three days. The first group of rat pups was sacrificed immediately after the last administration and the remaining groups were sacrificed at intervals of seven days. Results showed degenerative changes in the neurons of the hippocampus of the first group of rats. Less cell damage was observed in the groups that were sacrificed at later stages. The times of eye opening was advanced and abnormalities of posture and gait were observed in the initial stages of treatment and immediately after withdrawal of the drug. These abnormalities reduced as the animals aged. This suggests that the pathways of neural development may have reached normality by the third week of life.

KEYWORDS: Glucocorticoids, Hippocampus, Post Natal Development, Wistar Rat

The brain has been recognized as a complex target tissue for the genomic effects of steroid hormones which bring about long lasting alterations in brain structure and neurochemistry as well as changes in behaviour and endocrine functions (McEwen *et al* 1997). It is a target organ for the actions of hormones secreted by the gonads, adrenals and thyroid gland, and this sensitivity to hormones begins in embryonic life with the appearance of hormone receptor sites in discrete populations of neurons (McEwen 1987). Because the secretion of hormones is also under control by its neural and pituitary targets, the brain-endocrine axis during development is in a delicately balanced state that can be upset in various ways. Thus, any agent that disrupts normal hormone secretion can upset normal brain development. Exogenous substances that mimic the actions of natural hormones can also interfere with CNS development and differentiation. Glucocorticoids modulate higher brain functions (Sapolsky *et al* 2000a, De Quervain *et al* 2003), especially during brain maturation (Gould and Cameron 1996, Bakker *et al* 2001).

Dexamethasone is commonly administered to extremely low birth weight infants (ELBW) infants. This window of dexamethasone exposure in critically ill ELBW infants spans an extensive period of perinatal viability, ranging from 24 to 40 wk post conception. During this time, the human brain is undergoing significant structural and functional transformations, making it particularly vulnerable to external influences.

In contrast to the lack of demonstrated long-term benefit, there have been recent suggestions that the long-term neurodevelopment of babies who have received pharmacological doses of steroids may be substantially impaired, with a major increase in developmental disabilities and movement disorders (O'Shea *et al* 1999). Although there is considerable evidence detailing the effect of glucocorticoids in adult humans and animals, relatively less is known about the effects in children (Heffelfinger and Newcomer 2001).

The study was to investigate the effect of administration of glucocorticoids on the development of the hippocampus of rat neonates, and the long term effect of the drug on neural development.

MATERIALS AND METHODS:

Animals: 15 adult Wistar Rats were used in this work. The rats were purchased from the Animal House of the Department of Human Anatomy, Ahmadu Bello University, Zaria and also housed there. The animals were kept at room temperature in 12 hour light and dark cycles. They were allowed

free access to food and water. The animals were fed growers' mash obtained from Nassarawa Feeds, Kaduna. The animals were grouped into five groups of three rats each using the trio mating system (2 females: 1 male), (Flagel *et al.*, 2002). The female rats were separated from the male rats at approximately gestational day 18 and housed in pairs. The litter of each dam was considered as one group. The litters were culled so that each group consisted of six pups. The groups were designated as follows: Control groups (1A, 1B, 1C, 1D, 1E) and treatment groups (2A, 2B, 2C, 2D, 2E). The day of birth was considered day1.

Drug Administration And Dosage

4mg/ml of Dexamethasone was used as stock solution. The animals in the treatment groups were administered with 0.5mg/kg of dexamethasone for three days, (Flagel *et al*, 2002). The mode of administration was by subcutaneous injection. The control groups were administered with normal saline.

Experimental Procedure

Administration of dexamethasone began on day four (4). The animals were weighed daily and the appropriate dosages made up for each administration. Treatment groups 2A E were given daily injections of dexamethasone (0.5mg/kg) for four (4) days. The injections were discontinued after the third administration, that is, on day seven (7).

Treatment group 2A and Control group 1A were sacrificed one day after the third (3^{rd}) administration (day 7). Treatment group 2B and Control group 1B were sacrificed 7 days after cessation of administration (day 14). Treatment group 2C and Control group 1C were sacrificed 14 days after cessation of administration (day 21). Treatment group 2D and Control group 1D were sacrificed 21 days after cessation of administration (day 28). Treatment group 2E were sacrificed 28 days after cessation of administration.

Harvesting Of Tissues And Tissue Preparation For Microscopic Evaluation

At days 4, 7, 14, 21, and 35 respectively the animals were anaesthetized in a suffocating chamber using chloroform. Afterwards the fur, skin and skull were removed to allow access to the brain. The brain tissue was removed and placed in Bouins' fluid. The brain tissue was fixed immediately after removal by placing it in Bouin's fluid. The tissues were processed using an automated tissue processor of the Department of Human Anatomy, A.B.U Zaria. Tissue sections of 5 microns were made using a rotary microtome. The tissues were stained using special silver staining (Glees stain and Hirano-Zimmerman stain) in order to demonstrate the histology of the hippocampus. Photomicrographs of all the groups were taken at x250 and x400 magnification.

Microscopic Examination Of Tissues

The results of the microscopic examination of the hippocampus cells of the animals in treatment group 2A revealed evidence of neurodegeneration characterized by clumping of nuclear chromatin material in the polymorphic layer. The pyramidal layer shows blurring of nuclear outline, nuclear membrane disruption, pyknosis and karyolysis of nuclear chromatin as well as clumping of nuclear chromatin in the molecular layer. As shown in plate 2.

Plate 4 shows the histology of the hippocampus of the animals in treatment group 2B. There was improved histological structure of the hippocampal layer. The histological architecture of the cells in the polymorphic layer appeared intact. The nuclear outline and chromatin of some of the cells in the pyramidal layer appeared preserved while some of the cells show signs of cellular injury such as clumping and pyknosis of nuclear chromatin. Some viable cells were present in the molecular layer.

Plate 6 shows the histology of the hippocampus of the animals in treatment group 2C. The histological architecture of the cells in the polymorphic layer appeared intact. The nuclear outline and chromatin of some of the cells in the pyramidal layer appeared preserved with viable cells present exhibiting clear and distinct nuclear outline. Viable cells were present in the molecular layer and much of the histology is preserved. There was evidence of dendritic organization and axonal structure.

Plate 8 shows the histology of the hippocampus of the animals in treatment group 2D. The histological architecture of the cells in all the layers appeared intact. Viable cells were present in all the layers. Dendritic arborisation and axonal growth were observed.

Plate 10 shows the histology of the hippocampus of the animals in treatment group 2E. The histological architecture of the cells in all the layers appeared intact. Viable cells were present in

all the layers. Axonal structure and dendritic arborisation were evident.

General Behaviour

Unsteady and staggering motions were observed in animals in treatment groups 2B 2E from day 6 and 7. This unsteady gait continued until the 13th -14th day. There after the gait was stabilized and coordinated and the animals moved without any visible difficulty. There were no such motions observed in the control groups. The animals in both control and treatment groups started to walk at roughly the same time, but the treatment groups showed some abnormality in walking such as unsteady movement. The treatment groups also exhibited a delayed tendency to explore their cages. Eye opening was observed on day 9 in most of the animals and by day 11 all of the animals were observed to have open eyes. One of the animals in treatment group 2B became blind in one eye on day 4. The second eye closed the next day. The animal died before sacrifice.

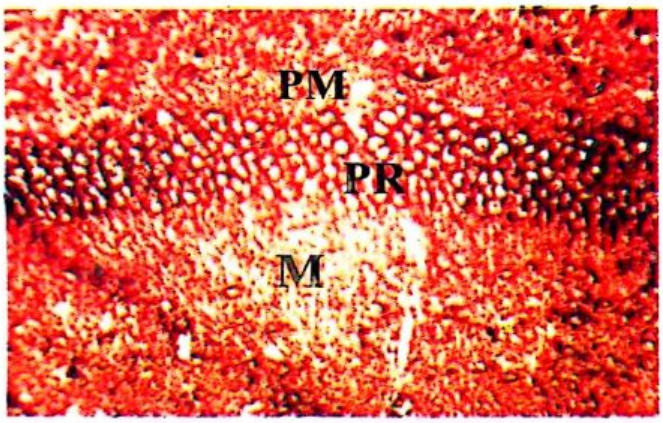

Plate 1: Micrograph showing the histology of the hippocampus of rat pup from control group 1A. Normal histological architecture of hippocampal layers (polymorphic layer (PM), pyramidal layer (PR), and molecular layer (MO)) was observed. Glees stain x 250

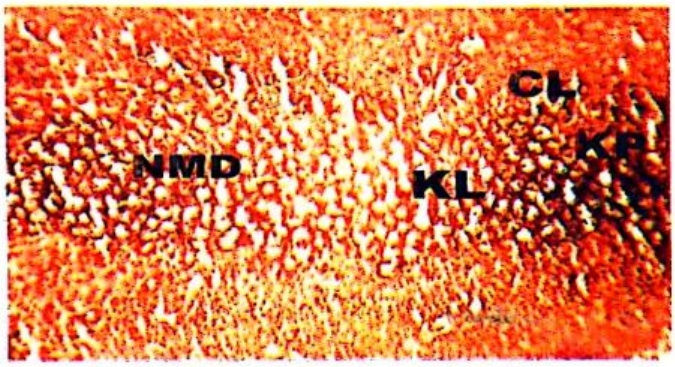

Plate 2: Micrograph showing the hippocampus of rat pup from group 2A Note clumping of nuclear chromatin (CL) in all the layers Also note pyknosis (PK), karyohexis (KH), karyolysis (KL) and nuclear membrane disruption (NMD). Glees stain x 250

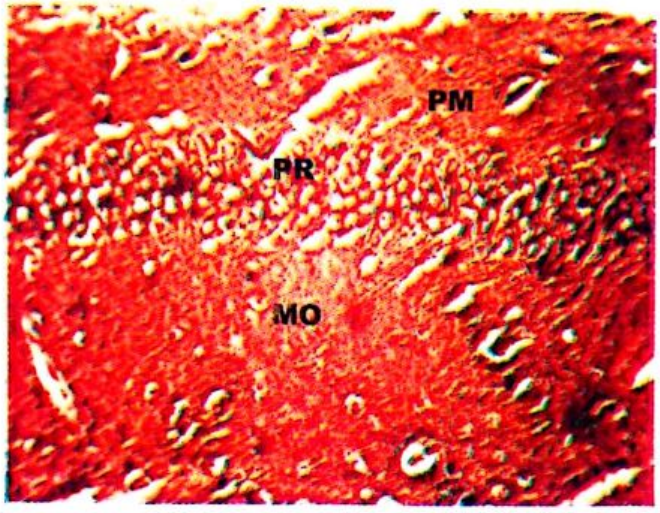

Plate 3: Micrograph showing hippocampus of rat pup from control group 1B Normal histological architecture of hippocampal layers (polymorphic layer (PM),pyramidal layer (PR), and molecular

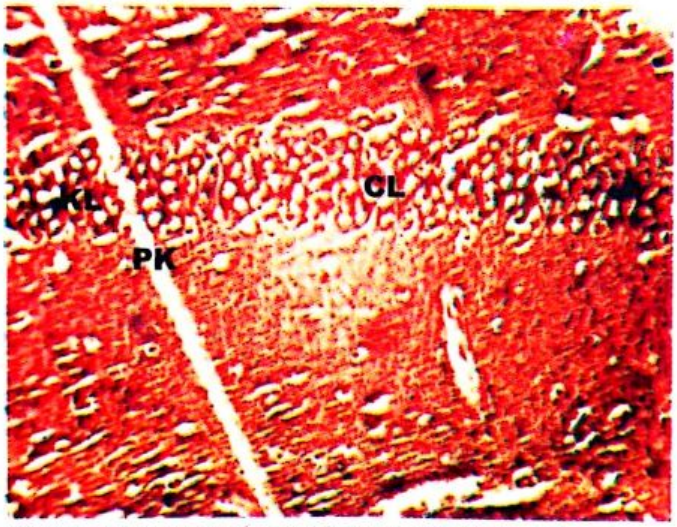

Plate 4: Micrograph showing hippocampus of rat pup from group 2B Some evidence of cell injury such as clumping (CL), pyknosis (PK) and karyolysis (KL) observed. Glees stain x 250.

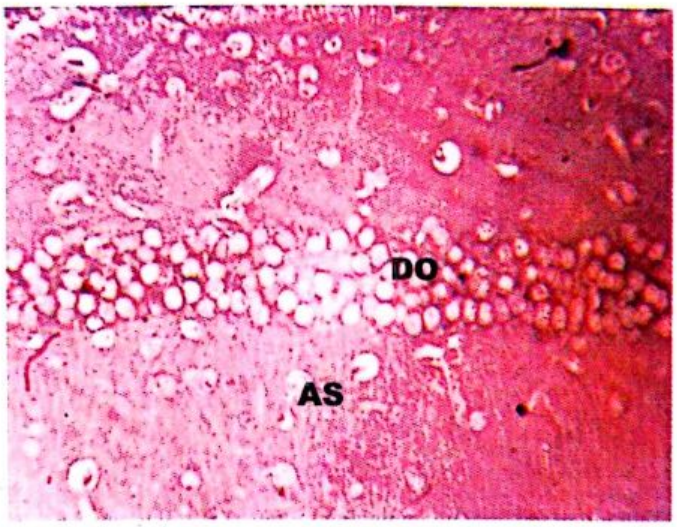

Plate 5: Micrograph showing hippocampus of rat pup from control group 1C Normal histological architecture of hippocampal layers (polymorphic layer (PM), pyramidal layer (PY), and molecular layer (MO)) was observed. Dendritic organisation (DO) and axonal structure (AS) observed. Glees stain x250.

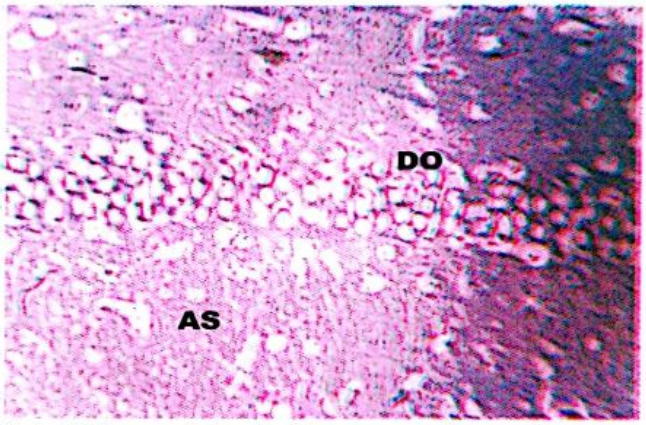

Plate 6: Micrograph showing hippocampus of rat pup from group 2C Preserved histological architecture of the hippocampu observed. Pyramidal cells showed adequate dendritic organization (DO) and axonal structure AS).

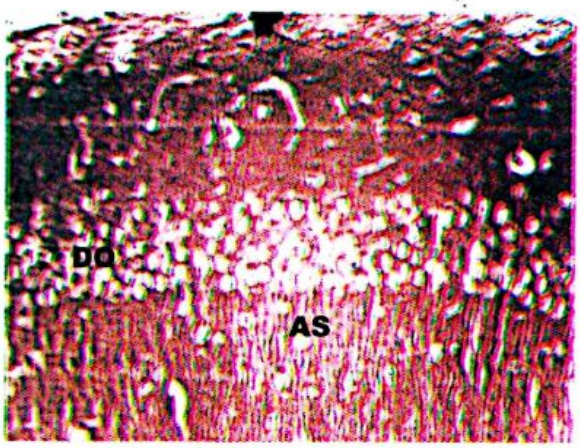

Plate 7: Micrograph showing hippocampus of rat pup from control group 1DWell established histological architecture of hippocampal layers was observed. Dendritic organisation (DO) and axonal structure (AS) are well established. Hirano-Zimmerman stain

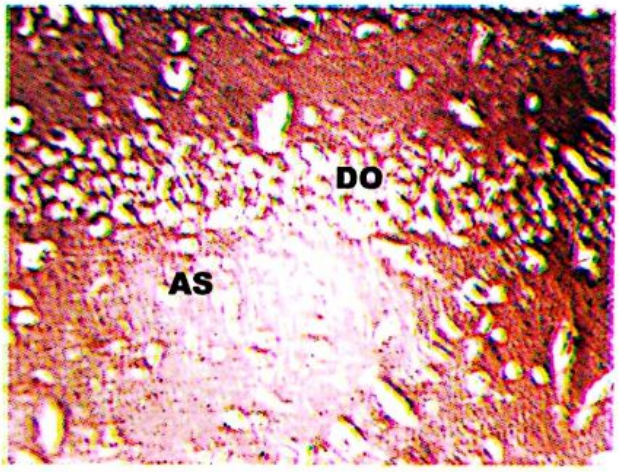

Plate 8: Micrograph showing hippocampus of rat pup from group 2D Preserved histological architecture of the hippocampus observed. Pyramidal cells showed adequate dendritic organization (DO) and axonal structure (AS). Viable cells (VC) present. Hirano-Zimmerman stain x 250.

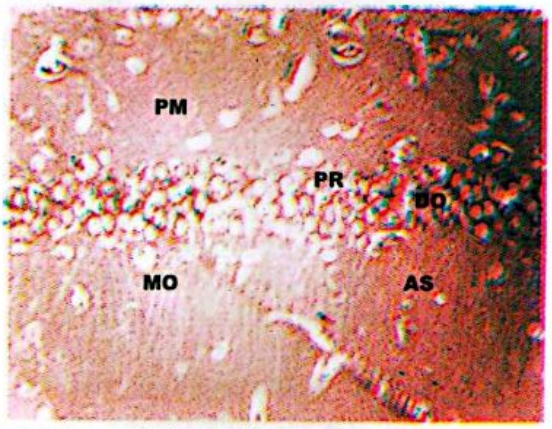

Plate 9: Micrograph showing hippocampus of rat pup from control group 1E Normal histological architecture of hippocampal layers (polymorphic layer (PM), pyramidal layer (PY), and molecular layer (MO)) was observed. Dendritic arborisation (DO) and axonal structure (AS) observed. Hirano- Zimmerman stain x250. Glees stain x250.

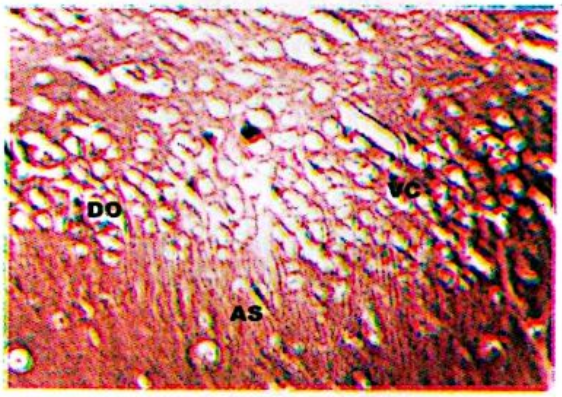

Plate 10: Micrograph showing hippocampus of rat pup from group 2E Preserved histological architecture of the hippocampus observed. Pyramidal cells showed adequate dendritic organization (DO) and axonal structure (AS). Viable cells (VC) present. Hirano-Zimmerman stain x 250.

DISCUSSION

Romijn *et al* (1991) based on a series of indices on brain development concluded that the rat's brain development at the 8th postnatal day is comparable to that of the human baby at term. Extrapolated, these ages can be considered analogous to the stage of brain development of foetuses or prematurely born babies at 27-34 week of gestational age. Animal models are beginning to emerge fit for investigating the neurological effects and possible mechanisms of perinatal glucocorticoids (Brabham *et al* 2000, Felszeghy *et al* 1996, Ferguson and Holson 1999). Although caution is necessary when extrapolating from animal models to the clinical setting, there is very little variation in the general sequence of brain growth between laboratory animals and humans (Dobbing 1974); the main differences being the timing of events that lead to spurts in brain growth.

In humans, neuronal proliferation is completed before the 24th week of gestation, exceptions being the cerebellum and dentate gyrus (Dobbing 1974). After this gestational age, glia continue to proliferate and oligodendrocytes maintain ongoing myelination, with a peak in brain growth occurring near term. In contrast to humans, rodents have their brain growth spurt after birth. It is estimated that in terms of brain growth rate, periventricular germinal matrix composition, neurochemical expression, electroencephalographic patterns, and synapse formation, at approximately postnatal *day* (PD) 7, the rat brain is roughly equivalent in development to that of a full-term human infant (38 to 40 week post conception (Dobbing 1974, Hagberg 1997). With this in mind the animals were administered daily injections of dexamethasone from day 4 to day 7.

Eye opening in normal rats occurs between the ages of 12 to 14 days after birth (Pass and Freeth, 1993). This corresponds with the period of eye opening observed in the control group of animals. Eye opening in the treatment groups was observed earlier. Flagel *et al* (2002) and Grambergen and Mulder (1998) reported earlier eye opening periods for dexamethasone treated animals in their respective works. These data on developmental processes that are speeded up, whereas other processes develop abnormally after corticoid administration at early stages point to a complicated influencing of metabolic processes after steroid administration during development (Uno *et al* 1990).

The staggering and unsteady motions observed in the treatment group correspond with the findings of Grambergen and Mulder, (1998). Locomotion in both experimental groups was characterized by a postural tremor and an abnormal posture during walking from the 9th until the 15th day. Although the walking pattern after this age became fluent, the gait width remained abnormally increased until the 20th day. In animal research it has repeatedly been reported that steroid treatment during neuro-ontogeny leads to abnormalities in brain development. In addition, abnormal motor behaviour, such as hyperactivity and impaired passive avoidance reactions, were demonstrated after postnatal administration of corticosterone from the 2nd until the 14th day after birth (Howard, 1968). Flagel *et al* (2002) observed that early neurological deficits observed in dexamethasone treated animals were no longer evident by day 20 suggesting that the pathways relevant to organization of reflexes and behaviour reached acceptable criteria of normalcy by the time of weaning.

This study revealed degenerative changes in the neurons of all the layers of the hippocampus caused by early dexamethasone administration These findings coincide with those of Howard (1968) who reported that corticosterone given to mice between 2 and 14 days of age interfered with synthesis of DNA, RNA, and protein in the brain and produced irreversible reductions in brain size weight, and cell numbers that persisted throughou most of the life span. Although neurones in mos areas of the human brain stop dividing by the third trimester, neurones in the hippocampal dentate gyrus continue to mature long after term and therefore remain vulnerable to adverse influences The mechanisms by which steroids reduce brain growth remain unclear but probably include inhibition of growth factors and facilitation o apoptosis (Riva *et al.*, 1995).

Steroid treatment during critical periods o brain development may impair myelination and brain cell division, resulting in longer tern behavioural effects (Weichsel, 1978). Single dose of dexamethasone given to rats on postnatal day 4 o 7 were associated with subsequent behavioura disturbances, reduced cerebellar weight, and impairments in spatial learning and moto coordination (Benesova and Pavlik, 1989).

These results also agree with Sapolsky *et al.* (1985) (1990) and Woolley *et al.*, (1990) who state that prolonged exposure to and excessiv glucocorticoids (GC) induced pyramidal and dendritic atrophy with neuronal loss in th hippocampus.

Interestingly, there was some evidence o reversibility of the hippocampal damage in th animals that were sacrificed at later stages. Thi agrees with Sousa *et al.*, (2000) and Starkman *et a* (1999) who reported that some form of reversibilit of the hippocampal atrophy and neuriti degenerative changes was reported both in roder and human studies. This may be due to the fact th hippocampus of the animals was still in th developmental stages and new nerve cells were sti being produced.

In conclusion, administration o glucocorticoids to neonates may result in advers effects on the neurological development neonate

Also behavioural deficits may become apparent later in life. Glucocorticoids may also lead to adverse effects on the development of the hippocampus and on spatial memory.

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