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Cytoarchitecture and brain-derived neurotrophic factor immunolocalisation in the cerebellar cortex of African grasscutter (*Thryonomys Swinderianus*)

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Abstract

Introduction: The study described the lamina organization and immunolocalisation of brain-derived neurotrophic factor in the cerebellar cortex of the African grasscutter, at defined postnatal periods. **Materials and Method:** Brain samples extracted from African grasscutter neonates on postnatal day 3, juveniles on postnatal day 72 and adults on postnatal day 450 were prepared for routine histology and immunohistochemistry, using antibody specific to brain-derived neurotrophic factor. **Results:** On postnatal day 3, all the laminae typical of the concentric lamina organisation of the mammalian cerebellar cortex were evident, but, and external germinal layer was also observed. On postnatal day 72, and thereafter, the external germinal layer was no more evident. On postnatal day 3, the tree-like arrangement (*Arbor vitae*) of the cerebellum was not very striking, as the interlobular fissures were incomplete. On postnatal day 72, the *Arbor vitae* were better presented, as more lobules had been completely separated by interlobular fissures; although, there were some incompletely separated lobules, presented with interlobular fissural lines. On postnatal day 450, the lobules were distinct as the interlobular fissures separated all the vermal and hemispheric lobules. In all the postnatal periods, the granule cell layer was the most populated, while the Purkinje layer was a single cell line of Purkinje neurones. At all postnatal periods, strong immunoreactivity to brain-derived neurotrophic factor was observed in the Purkinje layer; the cell bodies and dendrites of all Purkinje neurones were immunoreactive; while the nuclei in neonate Purkinje neurons where not immunoreactive, the nuclei in the adults were immunoreactive. The cerebellar granule cells were not brain-derived neurotrophic factor immunoreactive, suggestive of their non-synthesis or loss of the synthesized protein, by anterograde axonal transport, to paracrine function. **Conclusion:** These findings and others were related to some behaviours of the African grasscutter, and compared with similar report in other rodents.

Keywords: Purkinje layer, external germinal layer, granular layer, African grasscutter, brain-derived neurotrophic factor.

1 Introduction

The histologic presentation of the mammalian cerebellar cortex is that of a layered structure. The lower layer has the presumptive white matter, followed by the granule cell layer and the Purkinje layer which contains the Purkinje neurons that separate the granule cell layer from the upper molecular layer (KOMURO, YACUBOVA, YACUBOVA et al., 2001). Granule cells found in the granule layer are among the smallest but most numerous neurons in the brain (LLINAS, WALTON and LANG, 2004). In the molecular layer, the basket and stellate cells provide inhibitory input to the Purkinje cells, with basket cells synapsing on the Purkinje cell axons and stellate cells onto the Purkinje cell dendrites (PURVES, AUGUSTINE, FITZPATRICK et al., 2001). The cerebellar Purkinje cell is one of the largest and most complex neurons in the mammalian nervous system (PURVES, AUGUSTINE, FITZPATRICK et al., 2001). The unusual anatomical organization of the Purkinje cell dendrite has been conserved phylogenetically, suggestive of the importance of the Purkinje cell morphology to its function. The Purkinje neurons were named after their discoverer, a Czech anatomist called Jan Evangelista Purkynĕ.

Although most neurones in the mammalian brain are produced prenatally, some parts of the brain retain the ability to produce neurones postnatal, a process termed postnatal neurogenesis. Brain-derived neurotrophic factor is the most active neurotrophin in the stimulation and control of neurogenesis in the postnatal animal (BENRAISS, CHMIELNICKI, LERNER et al., 2001), and has emerged as a key regulator of synaptic plasticity (COHEN, BAS ORTH, KIM et al., 2011). The positive reversal effect of brain derived neurotrophic factor in age-related diseases such as Alzheimer's disease was reviewed by Budni, Bellettini-Santos, Mina et al. (2016). Synthetic administration of brain derived neurotrophic factor has also been shown to promote the survival of neurones affected in Parkinson's disease, spinal cord injury (ANKENY, MCTIGUE,

GUAN et al., 2001) and Huntington's disease (ZUCCATO and CATTANEO, 2007).

Although the concept of postnatal neurogenesis has proved helpful in the treatment of neurological defects in humans, little work done is geared towards the identification of brain parts capable of regeneration in other mammals. Brain parts of the African giant pouched rat (OLUDE, OLOPADE and IHUNWO, 2014), African elephant (NGWENYA, PATZKE, IHUNWO et al., 2011) and Wister rats (GELFO, DE BARTOLO, GIOVINE et al., 2009) have been suggested as sites of postnatal neurogenesis. Unfortunately, no part of the African grasscutter brain has been tested for postnatal neurogenesis using antibody specific to brain derived neurotrophic factor.

Although the African grasscutter is a wild rodent, it has been integrated into the minilivestock industry in Nigeria, thus bred. It is also advocated as a potential laboratory animal (ASIBEY and ADDO, 2000). Unfortunately, the behaviour of the rodent is not well known, as the biology is still being studied. Advances in developmental biology have increased the understanding of developmental disorders affecting the brain, both as isolated anomalies and as part of larger malformation syndromes. The present study will therefore, provide researchers with a reference point of how the normal African grasscutter cerebellum develops, and identify parts of the cerebellum that expresses brain-derived neurotrophic factor in the rodent. Such information may be valuable in studying teratogenic brain defects, and, improve the current knowledge of veterinary neuro-embryology in the African grasscutter. Consequently, the aim of the present study was to describe the structural changes that occur in the cerebellar layers and immunolocalise brain-derived neurotrophic factor in the same cerebellar layers of the African grasscutter at postnatal developmental periods, compare it with similar reports in other rodents, and confer a morpho-functional paradigm.

2 Materials and Methods

2.1 Experimental animals and management

Twenty seven African grasscutters, comprising of 9 neonates of 3 days old, 9 juveniles of 72 days old and 9 adults of 450 days old were utilized for the study. They were purchased from a commercial grasscutter farm in Elele, Rivers State, Nigeria. Locally made wooden cages with adequate ventilation, and measuring 1.5 m x 1.5 m x 1.5 m were used to transport the African grasscutters, by road, to the Veterinary Histology Laboratory of the Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. In the laboratory, there were transferred to standard laboratory animal cages and acclamatised for 1 month before commencement of the experiment. During the pre-experimental period, the African grasscutters were physically examined under careful restraint in the cages. Apparently healthy ones were utilized for the study. They were fed twice daily, at 8.00 am and 6.00 pm, with fresh guinea grass (*Panicum maximum*), fresh cane grass (*Eragrostis infecunda*) and commercial rodent-pelleted concentrates. Drinking water was provided *ad libitum*. The feeding troughs and drinkers were sterilized daily using Milton® (Laboratoire Rivadis, Louzy, France; active ingredient: sodium hypochlorite 2% w/w). The cages were also swept and disinfected daily using Milton®, as well as a broad spectrum bactericidal, fungicidal and virucidal agent.

The experimental protocol was approved by the Ethical Committee of Ahmadu Bello University, Zaria, Nigeria. Management of the experimental animals was as stipulated in the Guide for the Care and Use of Laboratory Animals, 8th Edition, National Research Council, USA (NATIONAL..., 2011).

2.2 Brain extraction

The African grasscutters were sedated by intraperitoneal injection of 20 mg/kg Thiopental Sodium (Rotexmedica, Trittau, Germany) and immediately weighed using a digital electronic balance (Citizen Scales (1) PVT Ltd., sensitivity: 0.01 g). Thereafter, each animal was placed on a dissection table on a dorsal recumbency, and perfused, via the left ventricle, with 4% paraformaldehyde fixative, using the method of Gage, Kipke and Shain (2012). Immediately after the perfusion fixation, the head was separated from the rest of the body at the atlanto-axial joint, using a pair of scissors and knife. Thereafter, each skull containing the brain was obtained after skinning and stripping off all the facial muscles. Then, craniotomy preceded brain extraction. Specifically, brain extraction was performed in a caudo-rostral and dorso-ventral direction, using scalpel blades, thumb forceps, rongeur and a pair of scissors. The meninges and underlying blood vessels were gently removed to expose the intact brain. The cerebellum was separated from the rest of the brain by severing the cerebellar peduncles. The extracted cerebellum was submerged in a vial of the fixative for 24 hours. Thereafter, the samples were washed in phosphate buffered saline, and maintained in the same fluid for cytoarchitectural and immunohistochemical studies.

2.3 Cytoarchitectural study of the cerebellar cortex

The fixed samples of the cerebellum were trimmed and placed in labeled tissue cassettes. The cassettes were transferred into a semi-enclosed bench-top tissue processor TP 1020 (Leica Biosystems, Nußloch, Germany) which dehydrated the tissues in graded alcohol (70%, 80%, 90%, 100%, 100% and 100%) and cleared in toluene. The tissues were then infiltrated with molten paraffin wax (BDH Chemicals Ltd. Poole, England) at 50°C, blocked in paraffin and labeled. Coronal sections of 5 µm thick of the Cerebellar cortex were cut from the embedded tissues using Jung rotary microtome 42339 (Berlin, Germany) and floated unto adhesive charged slides. The slides were heated in an oven at 60 °C for 2 hours, deparaffinized in 3 changes of xylene and alcohol, each, and taken to water for rehydration. Every 4th, 5th and 6th coronal sections of each sample were floated unto adhesive charged slides. Every 6th section on the slide was reserved for immunohistochemistry, while every 4th and 5th sections were stained thus: every 4th section was stained with haematoxylin and eosin stain for general histological study and every 5th section was stained with cresyl fast violet stain for the Nissl substance. Slides were oven-dried for 15 minutes and cover-slipped with DPX as the mountant. The cytoarchitecture of the cerebellar cortex was studied microscopically, and photographed with a digital eyepiece (Scopetek® DCM500, Resolution: 5M pixels) attached to a light microscope (OLYMPUS® EUROPA GmbH, XSZ107BN, Hamburg, Germany). Photomicrographs were taken at x 40, x 100, x 250 and x 400 magnifications. *Nomina Anatomica Veterinaria* (INTERNATIONAL..., 2005) was used for nomenclature.

2.4 Immunolocalisation of Brain-derived neurotrophic factor

To immunolocalise brain-derived neurotrophic factor in the cerebellar cortex, the slides of every 6th serial coronal section of the cerebellar cortex were incubated in 100X Citrate Buffer (Abcam PLC., Cambridge UK; Product number: ab93678; pH: 6.0) at 65 °C, heated to 95 °C and maintained for 20 minutes, for antigen retrieval. Thereafter, the antigen retrieval solution containing the slides was placed on the bench for 20 minutes, to attain room temperature. The slides were placed in the immunohistochemistry staining chamber, washed with Tris buffered-saline and 0.05% Tween 20 (TBST 10-0028; Genemed Biotechnologies Inc., California USA; pH: 7.4) for 2 minutes and hydrogen peroxide block from a Rabbit Specific ABC/DAB detection IHC kit (Abcam PLC., Cambridge, UK; Product number: ab64261) was applied and incubated for 10 minutes at room temperature. Again, slides were washed in Tris buffered-saline and 0.05% Tween 20 for 2 minutes and protein block from the Rabbit Specific ABC/DAB detection IHC kit was applied and incubated for 10 minutes at room temperature. The slides were washed again in Tris buffered-saline and 0.05% Tween 20 for 2 minutes and the Anti-brain-derived neurotrophic factor primary antibody (Abcam PLC., Cambridge, UK; Product number: ab101748; Dilution: 1:1000) was applied to the slides and incubated for 60 minutes at room temperature.

After washing the slides in Tris buffered-saline and 0.05% Tween 20 for 2 minutes, biotinylated goat anti-rabbit secondary antibody from the Rabbit Specific ABC/DAB detection IHC kit was applied to the slides and incubated for 10 minutes at room temperature. Slides were rinsed in Tris buffered-saline and 0.05% Tween 20 for 2 minutes. Thereafter, streptavidin peroxidase from the Rabbit Specific ABC/DAB detection IHC kit was applied and incubated for 10 minutes at room temperature. The slides were rinsed in Tris buffered-saline and 0.05% Tween 20 for 2 minutes and 3, 3-diaminobenzidine chromogen plus diaminobenzidine substrate (1drop:1.5ml) from the Rabbit Specific ABC/DAB detection IHC kit was applied to the slides and incubated for 3 minutes. The slides were washed in Tris buffered-saline and 0.05% Tween 20 for 2 minutes and counterstained with haematoxylin for 30 seconds. Slides were dehydrated, oven-dried for 15 minutes and cover-slipped with DPX.

The slides were examined under the light microscope at final magnification of x 400. Positive immunostaining of brain-derived neurotrophic factor was observed as a brown cytoplasmic precipitate. For assessment of the immunostaining, a semi-quantitative scoring was performed by three independent observers. The number of immunoreactive cells was classified as: - = none, + = mild (positive cells constituted less than 10%); ++ = moderate (10-50% of cells were positive); and +++ = strong (more than 50% of cells were positive).

The manufacturer of the anti-brain-derived neurotrophic factor primary antibody (Abcam PLC., Cambridge, UK), stated in the product datasheet, that mouse, rats and humans are immunoreactive to the primary antibody, and the immunomarker is highly expressed in the cerebral cortex, among other tissues. Thus, the cerebral cortex of Wistar rat was harvested and treated with the same primary antibody, at the same time with the test slides, and used as the positive control. A negative control was developed to validate the positive results; some control slides of the Wistar rat cerebral cortex were not treated with the primary antibody, and served as the negative control.

3 Results

3.1 Cytoarchitecture of the cerebellar cortex on postnatal 3 (neonatal period)

On a coronal section of the cerebellar cortex on postnatal 3, an external germinal layer (Figure 1: Arrow head) was observed separating the outer molecular layer of two lobules. All the laminae typical of the concentric lamina organisation of the mammalian cerebellar cortex were evident. The laminae were the outermost molecular layer (Figure 1: ML), the Purkinje layer (Figure 1: black line arrow), the granule cell layer (Figure 1: GL), and the innermost layer of white mater (Figure 1: WL). The thickness of the granule cell layer was not uniform in all the lobes. It was thickest in the middle of some lobules, and thin at the end of the lobule, but thinnest in the middle of other lobules. Conversely, the thickness of the white mater layer decreased from the middle of each lobule to the end of the lobule, where the white mater layer was almost completely absent. The tree-like arrangement (*Arbor vitae*) of the cerebellum was not very striking on postnatal day 3 (Figure 1).

On a high magnification, the white mater layer was composed of numerous cells and very few neurites (Figure 2: arrow head in WL). These cells were visibly larger, but less numerous than the granule cells of the granule cell layer (Figure 2: arrow head in GL). The cells of the molecular layer were also less numerous than the granule cells (Figure 2: arrow head in ML). Thus, the granule cell layer was the most populated, while the Purkinje layer was made up of a single cell line of Purkinje neurons (Figure 2: Line arrow).

All the neurones of the different cerebellar layers were mature. The basket and stellate cells of the molecular layer were evident. There were small to medium-sized neurones, oval to round in shape, with branching dendrites. The stellate

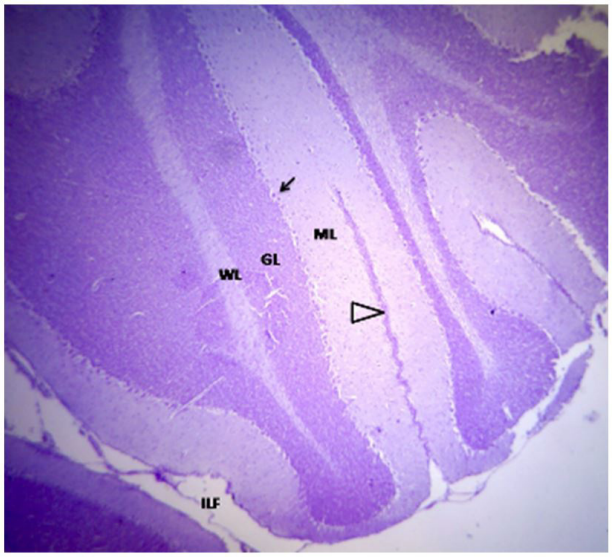

Figure 1. Coronal section of the cerebellar cortex of the African grasscutter on postnatal 3 ILF: Interlobular fissure; ML: Molecular layer; Black line arrow: Purkinje layer; GL: Granule cell layer; WL: White mater layer; Arrow head: external germinal layer. Cresyl fast violet stain x 40.

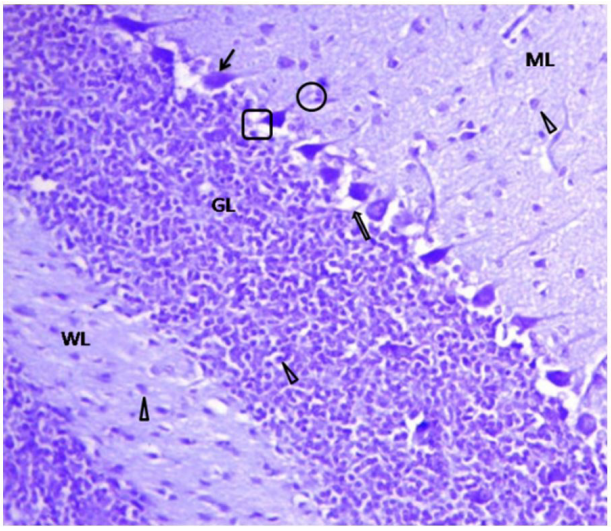

Figure 2. Photomicrograph of the neurones in the cerebellar cortex of the African grasscutter on postnatal day 3. ML: Molecular layer; Arrow head in ML: Stellate cells; Round shape in ML: Stellate-cell-Purkinje-cell synapse; Line arrow: Purkinje neurones; GL: Granule cell layer; Rectangular shape in GL: Granule-cell-Purkinje-cell synapse; Arrow head in GL: Granule cells; Block arrow in GL: Mossy fibres; WL: White mater layer; Arrow head in WL: neurones in the white mater layer; Cresyl fast violet stain, x 100.

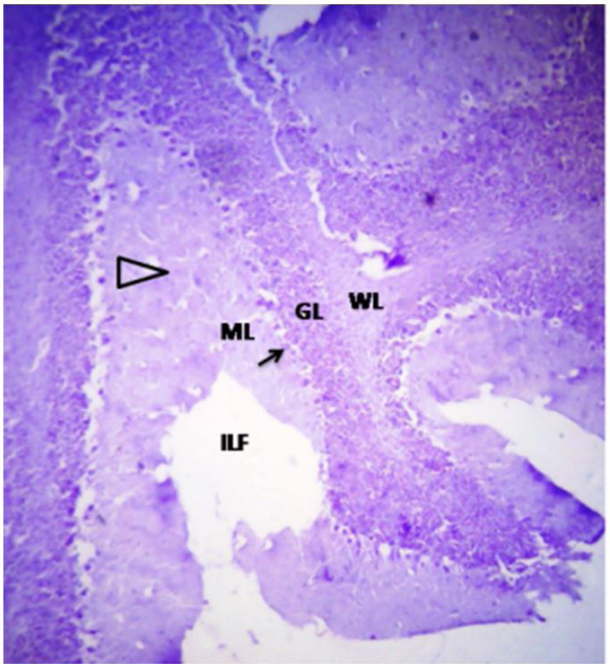

Figure 3. Coronal section of the cerebellar cortex of the African grasscutter on postnatal 72. ML: Molecular layer; Black line arrows: Purkinje layer; GL: Granule cell layer; WL: White matter layer; ILF: Interlobular fissure; Arrow head: Absence of external germinal layer in areas of incomplete interlobular fissure. Cresyl fast violet stain x 40.

cells of the molecular layer synapse with the dendrites of the Purkinje neurones (Figure 2: Round shape in ML). The Purkinje neurones were the largest of the neurones in all the layers. They were predominantly pyramidal multipolar neurones with well defined nucleus and numerous Nissl granules in the cytoplasm. Their dendrites extended into the molecular and granule cell layers. Granule cells were oval to round in shape and smaller in size than the Purkinje neurones. Within the granule cell layer, mossy fibres from the brainstem were visible (Figure 2: Block arrow in GL). The granule cells also formed synapse with the Purkinje cell dendrites (Figure 2: Rectangular shape in GL).

3.2 Cytoarchitecture of the cerebellar cortex on postnatal 72 (juvenile period)

The *Arbor vitae* were better presented on postnatal 72, as more lobules had been separated by interlobular fissures, unlike the case in the neonates. However, there were some incompletely separated lobules, presented with interlobular fissural lines (Figure 3: Arrow head in ILF).

The complete lamina organisation observed in the neonates was maintained in the juvenile, but the external germinal layer was no more evident at the period (Figure 3: Arrow head). The disposition of the varied thickness of the granule cell layer and the white mater layer was the same as observed in the neonates.

On a high magnification, the white mater layer was composed of less number of neurones and more tracts than those in the neonates (Figure 4: Arrow head in WL). The neurones were also smaller in size than those seen in the white mater layer of the neonates. The cells of the molecular layer were few, while the granule cells were the most numerous (Figure 4: Arrow

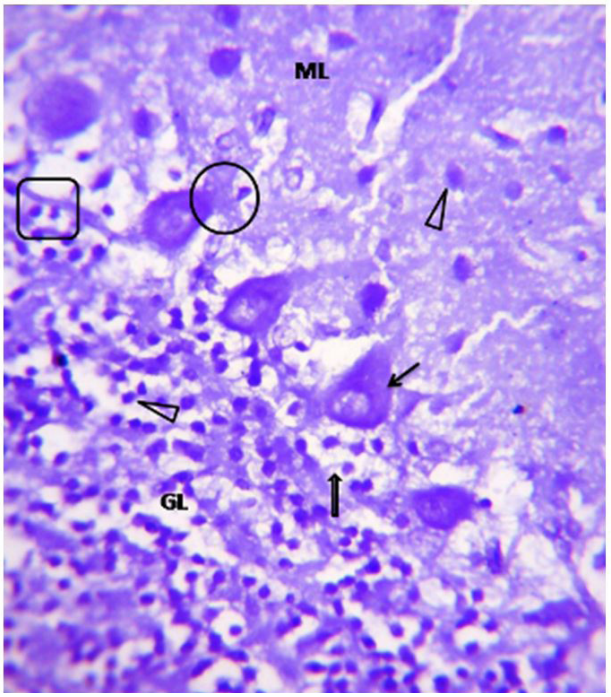

Figure 4. Photomicrograph of the neurones in the cerebellar cortex of the African grasscutter on postnatal 72. ML: Molecular layer; Arrow head in ML: Stellate cells; Oval shape in ML: Stellate-cell-Purkinje-cell synapse; Line arrow: Purkinje neurone; GL: Granule cell layer; Rectangular shape in GL: Granule-cell-Purkinje-cell synapse; Block arrow in GL: Mossy fibres. Cresyl fast violet stain x 400.

head in GL). The Purkinje layer was a single cell line of Purkinje neurones (Figure 4: Line arrow).

The stellate cells of the molecular layer were small to medium in size, oval to round in shape, and had branching dendrites. The stellate cells of the molecular layer synapse with the dendrites of the Purkinje neurones (Figure 4: Oval shape in ML). The Purkinje neurones were the largest of the neurones in all the layers. They were pyramidal multi-polar neurones with well defined nucleus and numerous Nissl granules in the cytoplasm. Their dendrites extended into the molecular and granule cell layers. Granule cells were oval to round in shape and smaller in size than the Purkinje neurones. Within the granule cell layer, mossy fibres from the brainstem were visible (Figure 4: Block arrow in GL). The granule cells also formed synapse with the Purkinje cell dendrites (Figure 4: Rectangular shape in GL).

3.3 Cytoarchitecture of the cerebellar cortex on postnatal day 450 (adult period)

On a coronal section of the adult cerebellar cortex, the vermal lobules maintained the tree-like arrangement (Figure 5). The lobules were distinct as the interlobular fissure, completely separated all the vermal and hemispheric lobules, unlike what was observed in the neonate and juvenile periods. On each lobule, the concentric arrangement of the cerebellar layers was maintained, with the molecular layer as the outermost layer (Figure 5: ML). This was separated from the granule cell layer (Figure 5: GL) by the Purkinje cells of the Purkinje layer (Figure 5: Black arrow). The innermost layer was the white matter layer (Figure 5: WL). The white matter layer varied in thickness along the entire concentric length of each lobule, such that there were thickest towards the middle of the lobule and almost absent at the tip of the lobule, where the granule cells occupied the entire space beneath the Purkinje cells. Conversely, the thickness of the granule cell layer was most at the tips, as they occupied the space of the white matter layers.

On a high magnification, the white mater layer was observed to be predominantly made of tracts (Figure 6: Black circle in WL). This was different from the observation in the neonate and juvenile periods. Very few neurones of the deep cerebellar nuclei were also observed in the layer (Figure 6: Arrow head in WL). On this magnification, it was also observed that the molecular and white mater layers had the least number of cells; the granule cell layer was the most populated, while the Purkinje layer was made of a single cell line of Purkinje neurones.

The neurones of the molecular layer were typically stellate cells. There were smaller than those of the neonates and juveniles, with branching dendrites. They synapse with the dendrites of the Purkinje neurones. The Purkinje neurones were the largest of the neurones in all the layers. Some were oval, most were pyramidal and multipolar. They had numerous Nissl granules; thus, very sensitive to the cresyl fast violet stain. Their neurites extended into the molecular layer to form the stellate-cell-Purkinje-cell synapse. The granule cell layer had the most numerous neurons, also known as granule cells. Granule cells were oval to round in shape and smaller in size than the Purkinje neurones. Within the granule cell layer, mossy fibres from the brainstem were visible. The granule cells also formed synapse with the Purkinje cell dendrites. They were the smallest in size, oval in shape. The granule cell density was higher in the adult than neonates.

3.4 Immunolocalisation of brain-derived neurotrophic factor in the cerebellar cortex at the different postnatal periods

The scoring of brain-derived neurotrophic factor immunoreactive neurones in the different layers of the cerebellar cortex was represented in Table 1. Mild immunoreactivity was observed in the molecular layer at all postnatal periods (ML in Figures 7, 8 and 9). Strong immunoreactivity was observed

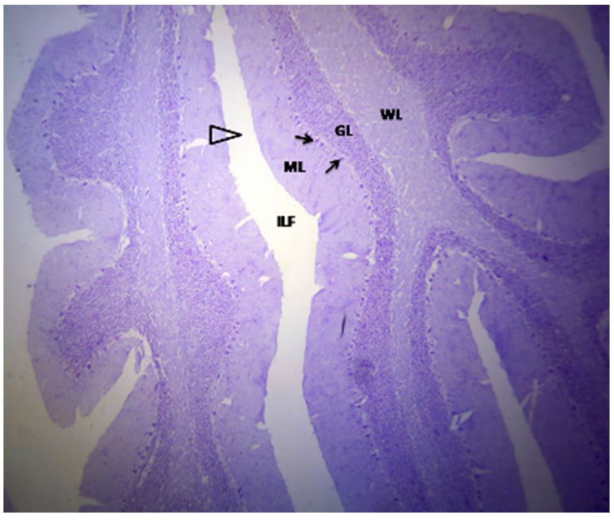

Figure 5. Coronal section of the cerebellar cortex of the African grasscutter on postnatal day 450 through Lobule I and Lobule II of the *vermis*. ML: Molecular layer; black arrows: Purkinje layer, indicated by the distinct Purkinje neurones; GL: Granule cell layer; WL: White matter layer; ILF: Interlobular fissure. Cresyl fast violet stain x 40.

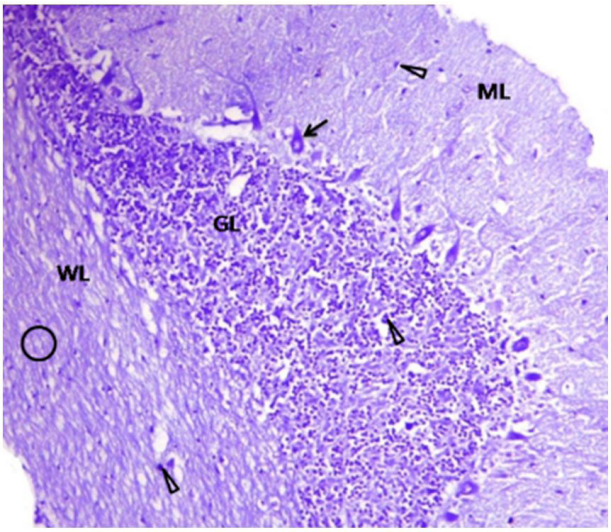

Figure 6. Photomicrograph of the neurones in the cerebellar cortex of the African grasscutter on postnatal 450. ML: Molecular layer; Arrow head in molecular layer: Neurones of the molecular layer; Line arrow: Purkinje neurones; GL: Granule cell layer; Arrow head in granule cell layer: Granule cell; WL: White mater layer with predominantly tracts (black circle) and few neurones (arrow head). Cresyl fast violet stain x 100.

Table 1. Quantitative analysis of brain-derived neurotrophic factor immunoreactive neurones in the African grasscutter cerebellar cortex at different postnatal periods.

Layers of Cerebellar Cortex	Scoring of Brain-derived Neurotrophic Factor Immunoreactive Cells at Different Postnatal Periods			
	Day 3	Day 6	Day 72	Day 450
Molecular layer	+	+	+	+
Purkinje layer	+++	+++	+++	+++
Granule cell layer	-	-	-	-
White mater layer	-	-	-	-

The number of immunoreactive cells was classified as: - = none, + = mild (positive cells constituted less than 10%); ++ = moderate (10-50% of cells were positive); and +++ = strong (more than 50% of cells were positive).

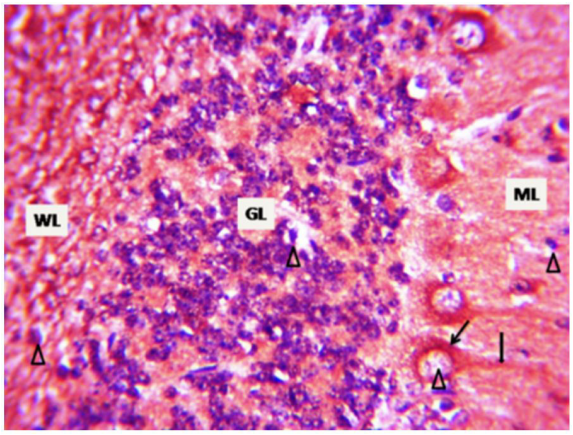

Figure 7. Immunolocalisation of brain-derived neurotrophic factor in the cerebellar layers of the African grasscutter on postnatal day 3. ML: Mild immunoreactivity in molecular layer with arrow head indicating non-reactive cells in the layer; GL: Non-reactive granule inter-neurones in the granule cell layer, indicated by the arrow head; Line arrow: Immunoreactive cell body of Purkinje neurones; Arrow head on Purkinje neurones: non-reactive nucleus of Purkinje neurone; Block arrow: Immunoreactive dendrite of Purkinje neurone; WL: Non-immunoreactive white mater layer, with arrow head indicating non-reactive cell in the layer; Magnification: x 400.

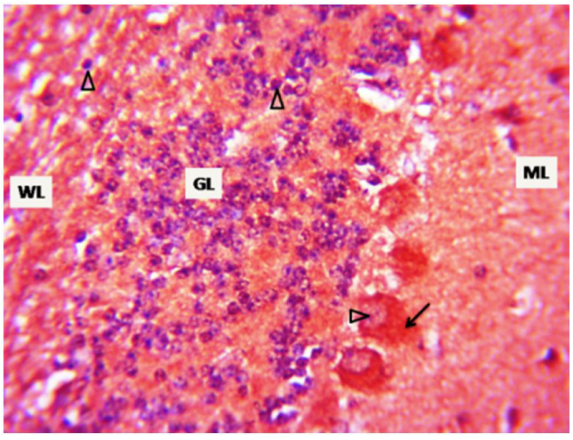

Figure 8. Immunolocalisation of brain-derived neurotrophic factor in the cerebellar layers of the African grasscutter on postnatal day 72. ML: Mild immunoreactivity in molecular layer; GL: Non-reactive granule inter-neurones in the granule cell layer, indicated by the arrow head; Line arrow: Immunoreactive cell body of Purkinje neurones; Arrow head on Purkinje neurone: Non-reactive nucleus of Purkinje neurone; WL: Non-immunoreactive white mater layer, with arrow head indicating non-reactive cell in the layer; Magnification: x 400.

in the Purkinje layer at all postnatal periods. The cell bodies (line arrow in Figures 7, 8 and 9) and dendrites (block arrows in Figures 7, 8 and 9) of all Purkinje cells were reactive, while the nuclei in neonates where not immunoreactive, the nuclei in the adult were immunoreactive. Negative immunoreactivity was observed in the granule cell (GL in Figures 7, 8 and 9) and white matter layers (WL in Figures 7, 8 and 9) in all the postnatal periods.

4 Discussion

The widely acceptable reason for the foliation pattern in mammalian cerebellum is the need to accommodate the large surface area of the cerebellum within a confined space of the neurocranium. Histological findings indicated the invagination of the Purkinje neurones into the deep granule cells at some points in the cerebellum at foetal day 90. This observation is important as those areas of invaginated Purkinje neurones represent the so called anchoring centres for the initiation of foliation in the cerebellum. Sudarov and Joyner (2007) used a combination of genetic inducible fate mapping, high-resolution cellular analysis and mutant studies in mouse to demonstrate that the first manifestation of anchoring centres in the cerebellum, when the cerebellar surface is smooth, is the slight invagination of the Purkinje cell layer. According to the authors, this invagination occurs due to the increased proliferation and inward thickening of the granule cell precursors, and at post-Purkinje invagination, the granule cells precursors within the anchoring centres become distinctly elongated along the axis of the forming fissure; thus, the inward folding of the outer cerebellar surface ensures. This process was observed in the mouse at about prenatal day 17.5 by Sudarov and Joyner (2007). The present study has established the process in the African grasscutter at prenatal day 90.

The external germinal layer was observed on postnatal day 3. By postnatal day 72, it was completely absent. In the mouse, cells of the external germinal layer reach maximum thickness on postnatal day 5-10 and completely disappear by postnatal day 20 (ANDRÆ, 2001). In normal Wistar rats, they completely disappear by postnatal day 21 (PATRO, MISHRA, CHATTOPADHYAY et al., 1997). According to Altman and Bayer (1997), the external germinal layer is an early transient layer that occurs during the postnatal cerebellar development, which covers the newly formed cerebellum, except the midline. Vriend, Ghavami and Marzban (2015) reported that the undifferentiated cells in the external germinal layer, which will eventually become the germinal cells, migrate from the external germinal layer radially, through the Purkinje cell layer, guided by Bergmann glia fibers, to the internal granular cell layer. Initially, Altman and Bayer (1997) reported that interneurones such as the basket cells, stellate cells and Golgi

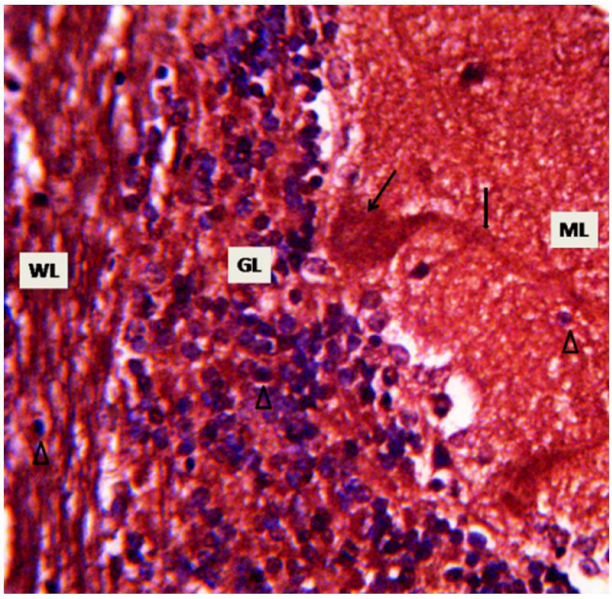

Figure 9. Immunolocalisation of brain-derived neurotrophic factor in the cerebellar layers of the African grasscutter on postnatal day 450. ML : Mild immunoreactivity in molecular layer with arrow head indicating non-reactive cells in the layer; GL: Non-reactive granule inter-neurones in the granule cell layer, indicated by the arrow head; Line arrow: Immunoreactive cell body of Purkinje neurones; Block arrow: Immunoreactive dendrite of Purkinje neurone; WL: Non-immunoreactive white mater layer, with arrow head indicating non-reactive cell in the layer; Magnification: x 400.

cells also originate from the external germinal layer. The presence of the external germinal layer by postnatal day 3 in the African grasscutter may indicate that the molecular and granule cell layers at the time were still in the formative stage.

On postnatal day 3, all the cerebellar layers were evident in the African grasscutter; the white matter layer was completely distinct from the granule cell layer. This is indicative of a mature cerebellum at birth. Sánchez-Villagra and Sultan (2002) reported similar feature in the neonatal cerebellum of other hystricognath rodents such as the Chinchilla (*Chinchilla lanigera*), river rat (*Myocastor coypus*) and Brazilian guinea pig (*Cavia aperea*). In their study, Sánchez-Villagra and Sultan (2002) categorized the cerebellar cortical layer appearance in rodents into stages 1 to 5. Stage 1 represented neonatal rodents in which a cerebellar anlage was present, and none of the later differentiating structures were discernable; while stage 5 represented rodents with clear separation of the granule layer from the white matter layer. It can be deduced from the present study that the African grasscutter belongs to stage 5, same as the above mentioned members of the same infraorder (*Hystricomorpha*). Sánchez-Villagra and Sultan (2002) also observed a strong correlation between cerebellar stage 5 and eye-opening at birth, a precocial condition typical of the African grasscutter. This is not unexpected, as precocial mammals depend greatly on a well-developed cerebellum for movement, motor co-ordination and balance, at birth and early neonatal period (NACHER, PALOP, RAMIREZ et al., 2000).

The four layered lamination of the cerebellum in the African grasscutter at postnatal period is typical of other rodents except the reeler mouse. The Purkinje cells formed a single cell-layer between the molecular layer and the granule cell layer in the adult African grasscutter, and in the reeler mouse, but only few Purkinje cells are arranged in a single cell line between the molecular and granule layers; some reside in the granule layer, and most are ectopically localized deep in the cerebellum (MIYATA, ONO, OKAMOTO et al., 2010). Katsuyama and Terashima (2009) attributed this observation to the absence of the Reelin gene in the cerebellum of the mutant mouse, as the gene is responsible for coding the single cell layer arrangement of the Purkinje cells.

The immunomarker, brain-derived neurotrophic factor was adopted for the present study due to its functional significance in the control of neurogenesis, especially dendridogenesis, axonal growth and synaptogenesis, as well as synaptic plasticity (CARTER, CHEN, SCHWARTZ et al., 2002; CARIM-TODD, BATH, FULGENZI et al., 2009; BUDNI, BELLETTINI-SANTOS, MINA et al., 2016). Several studies have emphasized the role of brain-derived neurotrophic factor in synapse morphology of Purkinje cells with other cerebellar neurones. Schwartz, Borghesani, Levy et al. (1997) observed atrophy of Purkinje cell dendrites on postnatal day 8 of mice, whose brain-derived neurotrophic factor was knocked out. The distribution of brain-derived neurotrophic factor in the cerebellar molecular layer and Purkinje cell body and dendrites in the present study is similar to the observation of Carter, Chen, Schwartz et al. (2002) in both wild-type and mutant mice on postnatal day 15. Similarly, Zhang, Li, Zou et al. (2007) observed brain-derived neurotrophic factor immunoreactive Purkinje cells in adult Rhesus monkey, although the immunoreactivity was less than that of the adult African grasscutter in the present result. The temporal non-immunolocalisation of brain-derived neurotrophic factor in the Purkinje cell nucleus in neonates, which became mildly expressed in the juvenile cell nucleus and finally, strongly localised in the adult cell nucleus is suggestive of retrograde conveyance of the protein from the cell body to the nucleus by simple diffusion or calcium dependent transcription as demonstrated by Cohen, Bas Orth, Kim et al. (2011). However, of interest in the present study is the age-dependent localisation of the immunomarker in the Purkinje nucleus. The reason for the finding is unknown.

Contrary to the present result, Hofer, Pagliusi, Hohn et al. (1990) observed moderate reactivity and Zhang, Li, Zou et al. (2007), observed strong reactivity of brain-derived neurotrophic factor in the cerebellar granule cells in the adult mouse and Rhesus monkey, respectively. The absence of brain-derived neurotrophic factor immunoreactive granule cells in the present study implies that either the cerebellar granule cells of the African grasscutter does not synthesize the protein, or the protein was immediately transported from the granule cells to other adjacent cells, by anterograde axonal transport. This is possible as brain-derived neurotrophic factor has been implicated in anterograde axonal transport from the site of synthesis to their target cells (CONNER, LAUTERBORN, YAN et al., 1997). This paracrine action of brain-derived neurotrophic factor is employed in the regulation of synaptic plasticity. Further analysis of brain-derived neurotrophic factor mRNA in the cerebellar granule cells of the African grasscutter is necessary as this will confirm or disprove the possibility of brain-derived neurotrophic factor synthesis by the cerebellar granule cells. This is because cells that were positive for brain-derived neurotrophic factor and its mRNA synthesize brain-derived neurotrophic factor; cells, negative for

brain-derived neurotrophic factor but positive for brain-derived neurotrophic factor mRNA also synthesize brain-derived neurotrophic factor, but the protein has been lost by the cell to paracrine function. Cells positive to brain-derived neurotrophic factor and negative to brain-derived neurotrophic factor mRNA do not synthesize brain-derived neurotrophic factor, but receive the pool of brain-derived neurotrophic factor by axonal transport from adjacent cells that actually synthesize the protein (CONNER, LAUTERBORN, YAN et al., 1997).

5 Conclusion

The present study has described the histologic appearance of the cerebellum of the African grasscutter on specific postnatal developmental periods, and compared the results with that of other rodents. Two key findings are the presence of the external germinal layer on postnatal day 3, which disappeared on postnatal day 72, and the incomplete lobulation by interlobular fissures on postnatal day 72. The study also exposed the spatial and temporal immunolocalisation of brain-derived neurotrophic factor in the cells of the African grasscutter cerebellar layers at the defined postnatal periods, with a negative result in the granule inter-neurones and strong immunolocalisation in the Purkinje neurones at all postnatal periods. Such information will be useful in future studies on the cerebellum of the African grasscutter, needed to fully comprehend the behaviour of the rodent and treat future neurological defects in the African grasscutter.

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