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Research Article

Some Aspects of Neuromorphology, and the Co-localization of Glial Related Markers in the Brains of Striped Owl (*Asio clamator*) from North East Nigeria

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Summary: The striped owl (*Asio clamator*) is unique with its brownish white facial disc and they are found in the north eastern part of Nigeria. Little is known in the literature on the basic neuroanatomy of this species. This study focuses on the histology and glial expression of some brain regions of the striped owl. Five owls were obtained in the wild, and their brains were routinely prepared for Haematoxylin and Eosin, and Cresyl violet staining. Immunostaining was done with anti-Calbindin, anti MBP, anti-GFAP, and anti-Iba-1 antibodies; for the expression of cerebellar Purkinje cells and white matter, cerebral astrocytes and microglia cells respectively. These were qualitatively described. We found that the hippocampal formation of the striped owl, though unique, is very similar to what is seen in mammals. The cerebellar cortex is convoluted, has a single layer of Purkinje cells with profuse dendritic arborization, a distinct external granular cell layer, and a prominent stem of white matter were seen in this study. The astrocytic population in cerebral gray is similar, though lacking in many processes as is typical in protoplasmic astrocytes, while the microglia were not strongly stained. The few stained microglia cells did not, however, show any features of activation. The striped owl's brain reveals some conserved aspects of cellular neuroanatomy in both the avian and mammals that are typical in these species. More work is however needed particularly in age related differences in these structures. This is perhaps the first report of Calbindin immunostaining in the brain of the striped owl.

Keywords*: Striped Owl; Immunostaining; Purkinje cells; Astrocytes; Microglia; Dendritic arborization*

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INTRODUCTION

The Striped Owl is a unique nocturnal bird with frontally placed eyes, facial disk, binocular vision, filoplumes, and a head that can rotate at about 2700 due to a single occipital condyle (Poster and Graphics, 2013). They are a relatively large owl species with a prominent tuft of elongated feathers on the crown resembling ears (Thurber et al., 2009).

Owls are adapted for hunting due in part to the ability to fly almost silently, move very slowly, and have eyes that have the capacity for aiding in capturing of nocturnal prey (Marti, 1974). In addition, they exhibit specialized hearing functions that also aid in hunting (Konig et al., 1999; John, 2013). There are few literature reports on the histology of the brain of the owl. Abd-Alrahman (2012) described the histology of the cerebrum of the barn owl.

The striped owl in particular possesses sophisticated capacity for vocalization used for nocturnal hunting that is developed through a sophisticated learning (Knudson, 2002). There is however, a paucity of information on the neuroanatomy of the striped owl, though it is suggested that her hunting adaptation demands central nervous system co-ordination.

This work is a preliminary study of the neuroanatomy of the striped owl in Nigeria focusing on the histology of some brain regions, and immunohistochemical expression of glial related markers.

MATERIALS AND METHODS

Sample Collection: A total of five owls (3 males and 2 females) were used for this study. They were

obtained by local hunters in the North Eastern of Nigeria. All birds were weighed and rectal temperatures obtained. The birds were subsequently sedated with 50mg/kg of ketamine hydrochloride. All experiments were done based on guidelines of University of Ibadan Ethical Committee.

Thereafter, their intercostal spaces were dissected and they were intracardially perfused using 4% buffered formalin. The brains (Plate 1) were then removed and placed in the same fixative for 24 hrs, replaced in fresh solution for another 24hrs, and thereafter transferred to 0.2% sodium azide solution (preservative against bacterial growth) and kept at 4^0C until processed.

Fixation and Tissue Processing of Samples for Paraffin Embedding, and Staining: The brains were dehydrated in graded concentrations of alcohol for one hour each (70%, 80%, 90%, 100% I, 100% II). The alcohol was in 2 changes of xylene for 2 hours each. Infiltration and impregnation were done in 2 changes of molten paraffin wax for 1 hour each with tissue embedded in the mould. Serial transverse and longitudinal sections of 5-µm thickness were prepared by using a HM330 Micron Microtome. For general histological examination, paraffin representative sections were stained by Hematoxylin and Eosin and Cresyl Violet as depicted by Suvarna *et al* (2013) and Ladagu *et al* (2020).

Immunohistochemistry: The antibodies used, and procedure of Immunohistochemistry (Ladagu *et al*) are as stated in Table 1.

Sections of the paraffin blocks were de-waxed and immediately rehydrated and immersed in distilled water. Retrieval of antigen was done using 10- mM citrate buffer (pH = 6.0) for a duration of 25 min with subsequent peroxidase quenching in 3% H2O2/methanol. After blocking in 3% H2O2/methanol, all the sections were blocked in 2% milk for 1 h after which they were probed with the following antibodies: Anti-GFAP (Glial Fibrillar Acid Protein) Rabbit Polyclonal, 1: 700, Dako Denmark), Purkinje cell aborization (Anti-Calbindin, 1:12,000, Abcam, USA), microglial morphology (Anti-Iba-1 Rabbit Polyclonal antibody, 1:500, Abcam, USA) and myelin expression (Anti-MBP (Myelin basic Protein) 1:500, Abcam, USA) at varying time points after due optimization at 4 °C. The sections were incubated for 2 h at room temperature using the appropriate biotinylated secondary antibodies (diluted 1:500; following Vector Labs' protocols) after washing. Avidin-biotin-peroxidase solution (ABC kit, Vectastain, Vector Labs, USA) were used afterwards for sections' reaction, and 3, 30-diaminobenzidine (DAB) was finally used as chromogen as instructed in manufacturer's protocol.

Experiments were done based on the ethical guidelines of the University of Ibadan for standard care and use of animals in research. All images were obtained using a bright field Leica DM 500 Microscope with in-built camera (Leica Microsystems, Wetzlar, Germany).

Table 1
List, type and dilution factor of antibodies used for immunohistochemistry

Name and Target of Primary Antibody	**Primary antibody**					**Biotinylated secondary antibody**
	Supplier	**Origin**	**Dilution**	**Incubation**	**Antigen retrieval**	
Anti-Calbindin (Purkinje cell aborization)	Abcam, USA	Rabbit	1:12,000	16hrs	Heat-induced epitope retrieval (HIER)	Anti-rabbit
Anti-GFAP (Astrocytic morphology)	Dako Denmark	Rabbit	1: 700	16hrs	Heat-induced epitope retrieval (HIER)	Anti-rabbit
Anti-iba 1 (Microglial morphology)	Abcam, USA	Rabbit	1:500	32hrs	Heat-induced epitope retrieval (HIER)	Anti-rabbit
Anti-MBP (Myelin expression)	Abcam, USA	Rabbit	1:500	16hrs	Heat-induced epitope retrieval (HIER)	Anti-rabbit

RESULTS AND DISCUSSION

The images obtained from this study are presented in Plates 1 - 8.

Animals without a "six-layered" cerebrum, like the owl, display complex intelligence and behaviour, tool utilization and skills comparable to other mammals (Güntürkün and Bugnyar, 2016; Karten, 2015). Recent reports have suggested that the "six-layered" cerebrum has evolved from a common ancestor with a "three-layered" cortex as typified by the hippocampus of varying animal species (Güntürkün et al., 2017; Shepherd and Rowe, 2017).

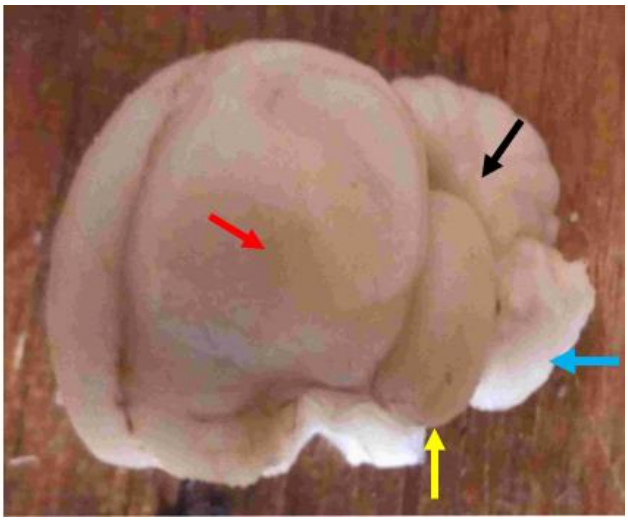

Plate 1:
Photograph of a whole brain of a striped owl, rostrolateral view. Red arrow, Cerebrum; yellow arrow, Rostral Colliculi; black arrow, Cerebellum; blue arrow: Medulla

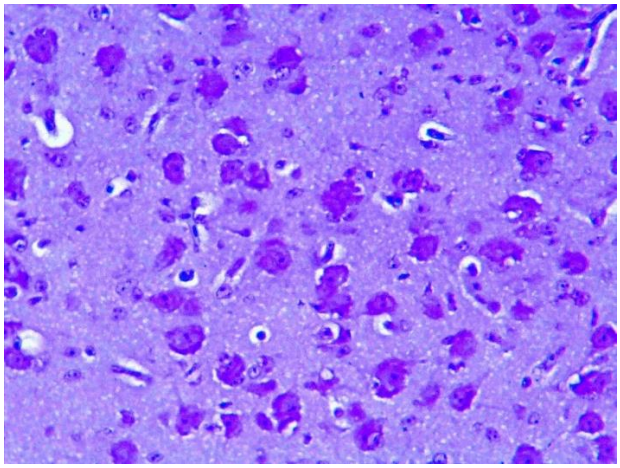

Plate 2:
Photomicrograph showing cortical histology of the brain of the striped owl with polymorphic neurons, Cresyl violet x 400.

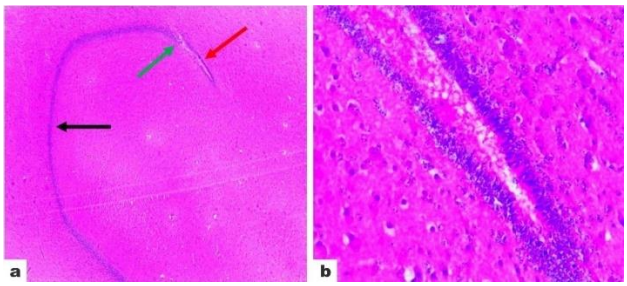

Plate 3:
a): Photomicrograph of the dentate gyrus (red arrow) and the CA1 region (black arrow), CA3 (green arrow) of the striped owl, H&E X 40. B). shows the Dentate Gyrus, H&E X 400.

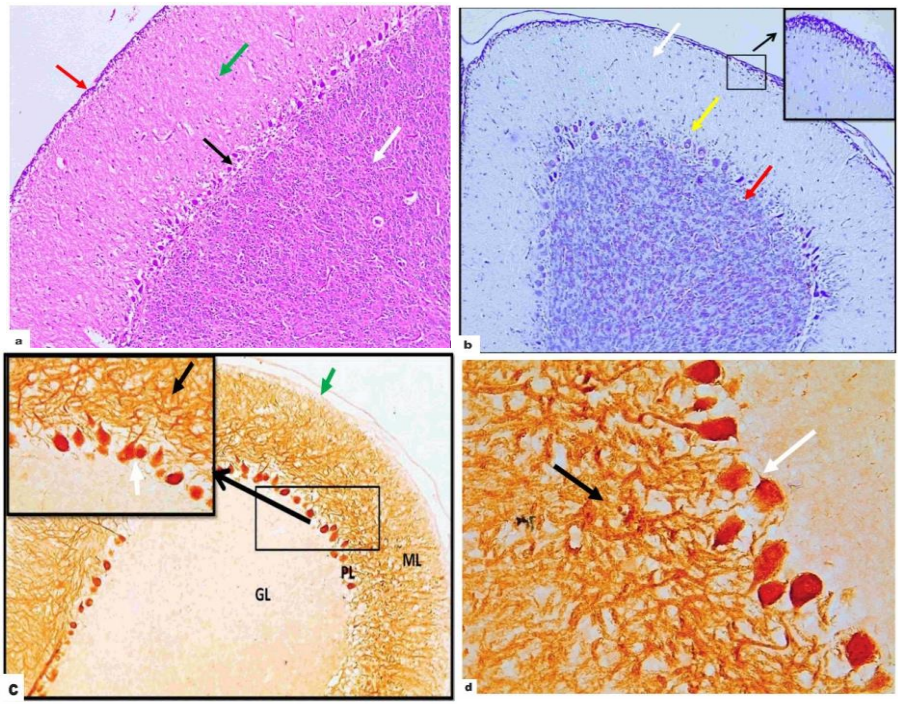

Plate 4:
Photomicrographs of the cerebellum of striped owl. a: Cerebellar cell layers: granular cell layer (white arrow), Purkinje cell layer (black arrow) and molecular cell layer (green arrow). Note the external granular layer (red arrow) H&E x 100. b: Cerebellar cortical layers of molecular (white arrow), Purkinje (yellow arrow) and the granular (red arrow) Cresyl violet x 100, inset x 400 magnifies the external granular layer. c: Purkinje cell layer (white arrow) and dendritic arborisation (black arrow), Anti-Calbindin immunostaining X 100. Note absence of immuno-localization in the external granular layer (green arrow). d: Purkinje cell layer (white arrow) and arborisation (black arrow) into the molecular layer, Anti-Calbindin X 400.

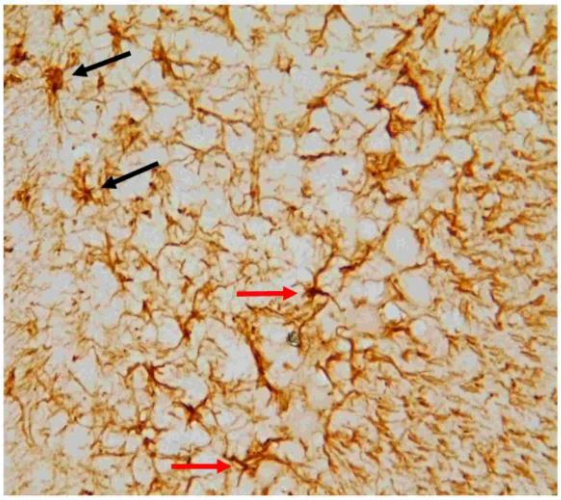

Plate 5:
Photomicrographs of the cerebrum of striped owl showing the fibrous astrocytes (black arrows) and protoplasmic astrocytes (red arrows), Anti-GFAP X 400.

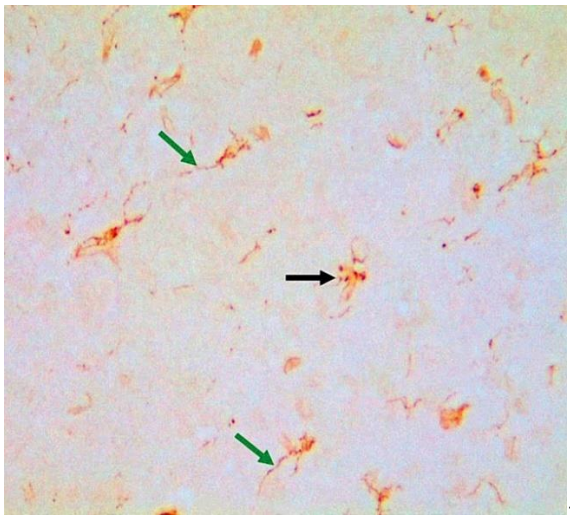

Plate 6:
Photomicrograph of the brain of striped owl showing resting microglia (black arrow) Iba1 X 400. Note the processes of the microglia (green arrows)

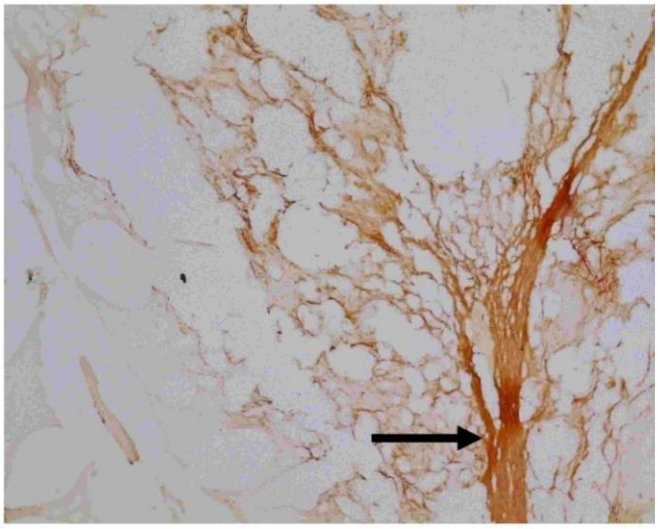

Plate 7:
Photomicrograph of the cerebellum of striped owl showing myelination of white matter (black arrow) MBP X 100.

Though looking unique, the hippocampus of the striped owl in this study (CA1, CA3, and Dentate Gyrus appearance) is similar to what is seen in mammals (Plates 2a and b)

Birds and mammals are the only species with variably convoluted cerebella (Iwaniuk *et al.,* 2006). In this study, the striped owl showed a typical cerebellar histomorphology of outer molecular, middle Purkinje cell and inner granular layers. The presence of the external granular layer (Plates, 4a, and 4b), however, suggests that the owls captured are relatively young. Butt *et al.*, (2014) described that only birds and mammals have granule cells which move in large numbers to form a transient external germinal layer; this cell layer has been shown to consist of stem cells in rodents and is Nestin positive (Bwala *et al.*, 2014). Calbindin localization in the cerebellum is known to clearly show Purkinje cells and dendritic arborization (Ioannidis *et al.*, 2019; Adebiyi *et al.*, 2020). This study shows the owl possess a thick and highly branched dendritic arborisation in the outer molecular layer (Plates 4c and d). Birds have been known to have a significant increase in dendritic arborization in the first 3 days of life (Mori and Matsushima, 2002). It is speculative to affirm that the high dendritic arborization may be a compensation for the reduced cerebellar hemisphere diminished by large rostral colliculi as seen in Plate 1. This is perhaps the first account of Calbindin expression in the brain of the striped owl and shows a lack of localisation of Calbindin to the external granular layer. This suggests that our optimized concentration can be adapted for further investigations on the cerebellum of the striped owl.

The GFAP (astrocytes) localisation in the brain of owls showed fibrous-like and protoplasmic astrocytes (Plate 4) in the cerebrum. Kalman and Pritz (2001) opined that mammals and birds exhibit similar general features in their glial architecture and GFAP distribution. There was a relatively weak expression of Iba-1 (microglia) staining in this study (Plate 5). It is still speculative to determine if they are resting microglia with no inflammatory activation. It is worthy of note, however, that the cells showed processes and devoid of amoebic isotypes as seen during inflammation (Folarin *et al.*, 2017). In addition, the MBP (myelin) expression seen in this study was also typical (Plate 6) as seen in rodents (Usende *et al.*, 2016).

In conclusion, this work has described a histological outlay of some parts of the brain of the striped owl, including the localization of glial related aspects of the brain, showing that some conserved aspects of cellular neuroanatomy in avian and mammals are so typical in these species. In this study, we report to the best of our knowledge, the first attempt at Calbindin localization in the cerebellum of the owl. More work is however

needed particularly in age related differences in all these structures.

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