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Comparative Morphometry and Histological Studies of the Cerebellum of Catfish (*Clarias gariepinus)* and Tilapia (*Oreochromis niloticus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AD designed the study, wrote the protocol. Author SAH wrote the first draft of the manuscript. Author AAI managed the literature searches, analyses of the study performed the dissection and histological analysis. Authors AD and SAS managed the experimental process. Author MAM identified the species of fish and manage the statistical tool. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The cerebellum is the major organ responsible for balance and equilibrium of the body and also coordinates the muscular tone. There is dearth of information on the comparative morphometric and histological organization of the cerebellum of both Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*).
Aims: This study is aimed at studying the gross, morphometry and neurohistologic organization of the cerebellum of Catfish and Tilapia.
Materials and Methods: A total of twenty (20) apparently healthy adult fish were Used. The fish were bred and raised in captivity with known medical records, identified and characterized at the

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fisheries department of Usmanu Danfodiyo University, Sokoto. Both the Catfish (*Oreochromis niloticus)* and Tilapia (*Clarias gariepinus)* were dissected to remove the brain.

Results: Gross, morphometry and histological differences of the cerebellum of the two species were studied. Catfish *(Clarias gariepinus)* brain was observed to be an elongated organ with distinct section situated just under roof of the skull. The anterior end of the cerebellum was closely associated with the caudal poles of the cerebral hemisphere, while the posterior end closely association with the pons and medulla. Catfish *(Clarias gariepinus)* brain was observed to be elongated organ with distinct section situated just under the skull roof. In *Oreochromis niloticus* the anterior end of the cerebellum was closely associated with the caudal poles of the cerebral hemisphere, while the posterior end being in close association with the pons and medulla. The cerebellum was covered by a transparent layer which invaginated into the fissures between the folia and also showed gyri and sulci. The cerebellum was observed to be connected with the midbrain rostrally and the medullar oblongata caudally via the peduncles. The cerebellum of both the species presented common anatomical features but exhibited some variations so far their gross and histological morphology.

Conclusion: This study shows the morphometric and histomorphological differences in the cerebellum of Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*) which could have contribute to the variation in their swimming pattern.

Keywords: Clarias gariepinus; Oreochromis niloticus; comparative anatomy; cerebellum.

1. INTRODUCTION

The cerebellum is the major organ responsible for balance and equilibrium of the body and also coordinates the muscular tone [1,2]. Different vertebrate fish possess different swimming pattern in the water pool [3,4]. Catfish swim by slithering its fins, and serpentine (snake-like) motion, whereas, Tilapia propels themselves by the action of their fins without great body motion [3,5,6]. The difference in the swimming pattern of these species of fish is the basis of this study. We hypothesized that there are differences in the morphological and histological organization between the cerebellum of Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*) which may account for the seemingly different swimming pattern. In addition, there is a dearth of information on the comparative morphometric and histological organization of the cerebellum of both Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*). An attempt was made to find out the gross anatomical and histomorphological variations of the cerebellum of Catfish and Tilapia, which may help in understanding the neurophysiology and functional anatomy of these species as it relates to their swimming patterns.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of twenty (n=20) apparently healthy adult fishes (10 each of Catfish and Tilapia) were used, which comprised of 5 males and 5 females for each species. The samples were obtained from Bado area of Wamako local government of Sokoto state where they were bred and raised in captivity with known medical records, identified and characterized at the fisheries department of Usmanu Danfodiyo University, Sokoto. These fishes were transported in plastic aquaria to the Veterinary Anatomy laboratory and humanely euthanasia in ice slurry immersion, with the consent of the standing committee on animal ethical and welfare of Usmanu Danfodiyo University, Sokoto to undertake the research study in fish.

2.2 Morphometry

All the fishes were weighed using Metlar Electronic (digital) weighing balance (Model: MT 2000) with the capacity/graduation of 1000 g/0.1 g and recorded in grams. The standard lengths of the fishes were measured in centimeter using a tape ruler. The Catfish (*Oreochromis niloticus)*, was dissected by making an incision between the upper and lower jaws and the frontal bone was reflected. The bony plate which covered the brain ventrally was crushed by the use of a needle holder following which the brain was exposed. In the Tilapia (*Clarias gariepinus*), the brain was exposed by an incision on the dorsolateral aspect of the head from the edge of the eye to the operculum, the bony structures were removed together with the operculum. The brains were examined *insitu* and photographs were taken using a digital camera (LG. 16 megapixels).

The brains were removed and the cerebellar were separated from the rest of the brain by cutting using a scalpel blade and cerebellar weights were taken using the same weighing balance.

2.3 Histology

The cerebellums were immersed in Bouin's solution for 48 hours. The fixed specimens were dehydrated in graded ethanol concentrations (50%, 60%, 70%, 80%, 90%, and 100%), cleared in two different concentration of xylene, and embedded in paraffin wax. Sections of about 4–6 μm thick were obtained with the help of rotary microtome and placed in a warm water bath. Tissue sections were collected from warm water bath and mounted on clean and dry glass slides and stained with Harris' Hematoxylin and Eosin (H&E) and viewed under light microscope from where photomicrographs were obtained with the aid of a digital camera attached to the microscope.

3. RESULTS

3.1 Morphological Studies

<u>3.1.1 Catfish *(Clarias gariepinus)*</u>

Catfish *(Clarias gariepinus)* brain was observed to be elongated organ with distinct section situated just under the skull roof (Plate 1a). The anterior end of the cerebellum was found to be closely associated with the caudal poles of the cerebral hemisphere, while the posterior end being in close association with the pons and medulla (Plate 1b). The cerebellar surface was found to be covered by a transparent layer which invaginates into the fissures between the folia and was found to have prominent gyri and sulci throughout the dorsal surface. The cerebellum was observed to be connected with the midbrain rostrally and the medullar oblongata caudally via the peduncles (Plate 1c). The sagittal section showed numerous folia separated by fissures. There was no observed morphological difference between the brains of male and female.

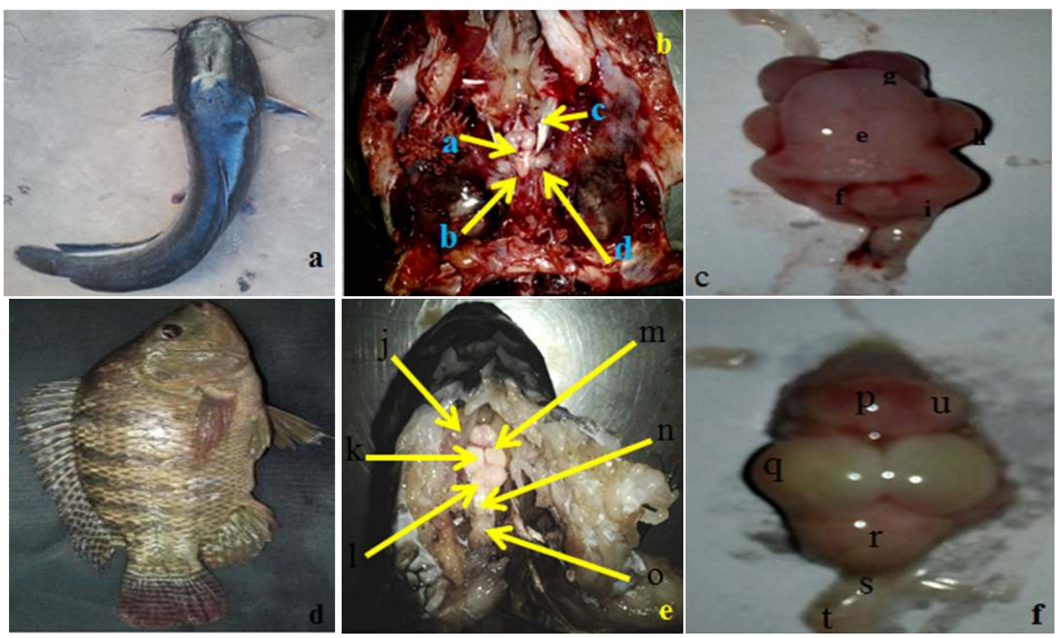

Plate 1. a. African Catfish (*Clarias gariepinus*). b. Photograph of the ventral view of the Catfish (*Claris gariepinus*) brain in situ (a) Optic lobe (b) Cerebellum (c) Hypothamus (d) Medula oblongata. c. Photograph of dorsal view of the Catfish (*Clarias gariepinus*) brain (e) Telencephalon (cerebrum), (f) Cerebellum (g) hypothalum, (h) optic lobe (i) medulla oblongata. d. Tilapia *(Oreochromis niloticus). e.* Photograph of the dorsal view of the brain of Tilapia (*Oreochromis niloticus) in situ* (j) Epithalamus (k) Cerebrum (l) Cerebellum (m) Hypothalamus (n) Pons (o) Medulla oblongata. f. Photograph of the dorsal view of the brain of Tilapia (*Oreochromis niloticus)* (p) Epithalamus, (q) Cerebrum, (r) Cerebellum, (s) Pons, (t) Medulla oblongata, (u) Hypothalamus

3.1.2 Tilapia *(Oreochromis niloticus)*

Tilapia *(Oreochromis niloticus)* brain is whitish and it's in close relation with the eye with the brain being enclosed in a curved bony plate while observed *in situ* (Plate 1d). The brain was observed to be ovoid in shape with distinct sections (Plate 1e). The anterior end of the cerebellum was found to be closely associated to the caudal poles of the cerebral hemispheres, with the posterior end in close association with the pons and medulla (Plate 1e). The cerebellar surface was found to be covered by a transparent layer which invaginates into the fissures between the folia and also showed gyri and sulci. The cerebellum was observed to be connected with the midbrain rostrally and the medullar oblongata caudally via the peduncles (Plate 1f). There was no observed morphological difference between the brains of both sexes.

3.2 Morphometry

3.2.1 Catfish *(Clarias gariepinus)*

The mean standard length, mean head length, average body weight, average brain weight, average cerebellar weight and relative cerebellar index were 34.69±0.86 cm, 8.84±1.15 cm, 245±22.73 g, 0.62±0.05 g, 0.25±0.05 g, and 0.10±0.02% respectively as shown on Table 1 and there were statistical significant difference (*p<.05),* with the values obtained from Tilapia species.

3.2.2 Tilapia *(Oreochromis niloticus)*

The mean standard length, mean head length, average body weight, average brain weight, and average cerebellar weight were 24.34±0.69 cm, 5.13±0.16 cm, 185.00±15.09 g, 0.15±0.02 g, 0.04±0.01 g, and 0.02±0.01% respectively as shown in Table 1 and there were statistical significant difference with the values obtained from Catfish.

Table 1. Mean standard length, mean head length, mean body weight, mean weight of the brain, mean weight of the cerebellum and relative weight of the cerebellum

Parameter	Catfish (n = 10)	Tilapia (n = 10)
SL (cm)	34.69±0.86^a	24.34±0.69^b
LH (cm)	8.84±1.15^a	5.13±0.16^b
BW (g)	245.00±22.73^a	185.00±15.09^b
WB (g)	0.62±0.05^a	0.15±0.02^b
WC (g)	0.25± 0.05^a	0.04±0.01^b
RWC (%)	0.10±0.02^a	0.02±0.01^b

Mean with different Superscript (a, b) along the rows have significant statistical difference (p<.05).
Key: SL: Standard length, LH: Head length, BW: Body weight, WB: Weight of the brain, WC: Weight of the cerebellum, RWC: Relative weight of the cerebellum

3.3 Histomorphology

3.3.1 Catfish *(Clarias gariepinus)*

The three strata- an outer molecular layer, intermediate layer of Purkinje cells and an inner deeper layer of granular cells that made up the cortex were observed in the cerebellar cortex of the Catfish. The molecular cells layer was observed to have a sparse population of neurons and stellate cells are found in the molecular layer as shown in Plate 2g. The Purkinje cells were found in a single row between molecular and granular cells layer as shown in Plate 2h. The thick granular layer resembles a clump of lymphocytes, and each granule cell had a spherical nucleus as shown on Plate 2h.

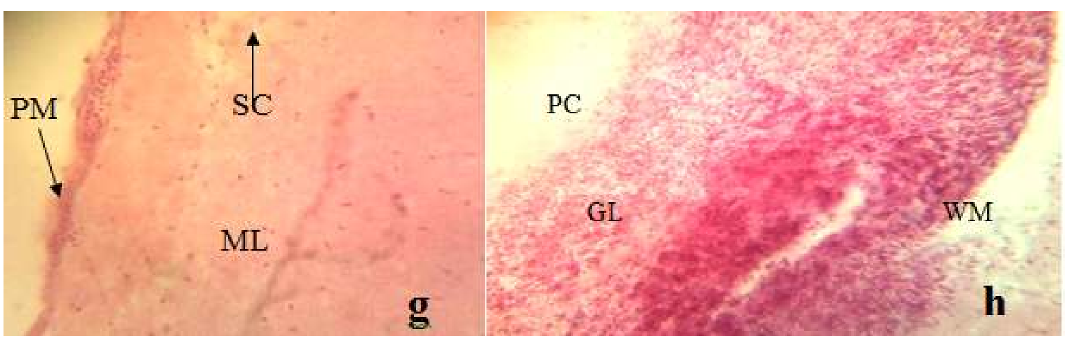

Plate 2. g. Photomicrograph of the molecular layer (ML), pia matter (PM) and Stellate cells (SC) of the cerebellar cortex of Catfish showing a sparse population of neurons (H & E) 400x.
h. Photomicrograph of the granule cells that made up the granular layer (GL) of the cerebellar cortex, single row of Purkinje cells (PC) and white matter (WM) in the Catfish (H & E) 400x

3.3.2 Tilapia *(Oreochromis niloticus)*

The neurons in the cerebellum of Tilapia species were uniformly distributed in both the folia and strata of the cerebellum. The granular layer which is present in Catfish is ill defined in the Tilapia species. Instead, the granule cells are widely distributed as observed in Plate 3.

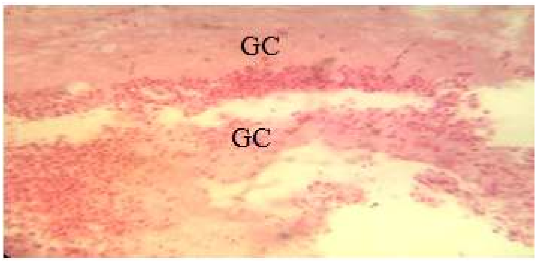

Plate 3. Photomicrograph of the cerebellar cortex of the Tilapia showing a sparse population of neurons (H & E), granule cells (GC) 400x

4. DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Discussion

The cerebellum of both the species presented common anatomical features but exhibited some variations in their gross and histological morphology. This finding is in agreement with [7], who stated that throughout all vertebrate genera the organization of cells and axons within the cerebellum had a similar and distinctive appearance.

The volume and weight of the cerebellum of Catfish was almost four times greater than that of Tilapia of same body weight. It's rounded in shape and its position was caudodorsal to the cerebral hemisphere and dorsal to the pons and medullar oblongata. The sulci which divide the surface of the cerebellum of both the species and the fissures that separated the inner lobes of the cerebellum were found to be finer (hardly visible) in the Tilapia and coarser in the Catfish. Externally the cortex was covered by pia mater in both species under this study. White mater was observed to have formed the inner bulk of the organ.

The molecular layer of the cortex contains thin myelinated neurons that originate within the layer, which is also in accordance with [8]. According to [8], the dendrite of Purkinje cells from the layer below are also seen in the molecular layer, but this was not observed.

A number of small star shaped cells found in a scattered manner close to the periphery of molecular layer represented stellate cells. This is also in agreement with [9], in fowl. Purkinje cells formed a layer between the molecular and granular cells layer in the Catfish. This is also in accordance with [7], who reported that Purkinje cells formed a single layer of large flask shaped cells at junction of molecular and granular layer in birds. This layer was not found in the Tilapia specie, whereas a number of large Purkinje cells were found around the granular cells layer. The granular layer was the innermost layer of the cortex and situated between the Purkinje cell layer and white matter that formed the medullar, this is in agreement with [9], in fowl. This layer was composed of numerous small granule cells together with nerve fibers. Granule cells have a relatively large nucleus. The cells of the granular layer appeared similar to lymphocyte because of large chromatic nucleus. The cerebellar white matter was observed to be featureless, moreover, its function as a germinal zone is controversial [10], although the structural and functional pattern of the brain is not distorted.

4.2 Conclusion

In conclusion, this study shows that there are morphometric and histomorphological differences in the cerebellum of Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*) which could contribute to the variation in their swimming pattern. This work could probably be the first morphological and histological studies on the brain of these species of fish, and the study is expected to provide background data for future research involving this and other parts of the brain of more fish species.

4.3 Recommendation

Further studies on the Histometry, Histochemical and Ultrastructural studies on the cerebellum of both Catfish and Tilapia species is recommended for more insight into the neurophysiology of their swimming pattern.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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