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BRAIN GROSS ANATOMY AND CEREBELLAR HISTOLOGY OF THE CATTLE EGRET (*BUBULCUS IBIS*)

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ABSTRACT

The structural parts of brain are similar in all vertebrates, but they differ in their complexity and organization. The avian brain for instance, is organised differently compared to mammals, with variations existing in the relative size and location of their structures. The cattle egret (*Bubulcus ibis*) is a cosmopolitan avian species native to Africa. Despite their economic importance as excellent sentinels in assessing environmental pollutants, there is a dearth of information on their neuroanatomy. Hence, we here investigated their gross morphological features and morphometric parameters, and the cerebellar histology. Twelve transcardially perfused juvenile cattle egrets were studied, with the body and brain weights and brain linear measurements considered. The brain weight constituted 0.46 % of the total body mass, with a strong positive correlation recorded between the body and brain weights (r=0.9204). Morphologically, the brain was lissencephalic, with the corpus callosum absent and the olfactory bulbs rudimentary with no discernible division into the olfactory bulb, olfactory tract and olfactory lobe. We observed prominent sagittal eminence and vallecula telencephali, as well as an obvious fovea limbica on the dorsal and lateral surfaces of the cerebral hemispheres, respectively. The diencephalic structures were completely covered by telencephalon, with the pineal gland occupying the polygonal space between cerebrum, optic lobe and cerebellum. The mesencephalic tectum appeared as a large oval bilaterally bulging structure with prominent optic tracts and constituted the bulk of the midbrain. There was a dorsal extension of the fourth ventricle into the cerebellum known as the ventriculus cerebelli and the cerebellar histology presented a persistent external granular layering suggestive of a potential for adult neurogenesis. Our data has added relevant literature on the cattle egret brain and could prove useful in comparative, developmental and evolutionary avian neuroanatomy.

Key words: avian; brain stem; cattle egret; cerebellum; cerebral hemispheres; neuro-morphometry

INTRODUCTION

The nervous system of vertebrates is divided according to functional or structural features. Functional classifications are the somatic and autonomic nervous system, and these are concerned with conscious and involuntary processes, respectively [9]. Meanwhile, structurally, the nervous system is divided into: the peripheral nervous system (PNS) comprising the cranial, spinal and autonomic nerves, and the enteric nervous system; and the central nervous system (CNS), constituted by the brain and spinal

cord [11]. The brain controls and regulates many important functions and is regarded as one of the most important organs of the body [50].

Comparatively, the structural parts of the brain are similar in all vertebrates, but they differ in their complexity and organization [45]. The avian brain, for instance, is organised differently compared to mammals, with variations existing in their structural sizes and/or locations [33]. Functionally, the avian nervous system obtains information through sensory receptors about their environment, analyse, respond and store the processed information and, also it coordinates the motor impulses to skeletal muscles as well as to the viscera [7, 27].

The cattle egret (*Bubulcus ibis*), an avian species of the heron, is found all over the world, with a least concern conservation status [48]. They belong to the order Pelecaniformes, family Ardeidae. They are native to Africa with a wide distribution in tropical and subtropical African regions including Nigeria, and are mostly found in dry grassy habitats, in association with wildlife and livestock [32]. The remarkable migration of the cattle egret from its origins in Africa to its present geographical range including Asia, the Americas and Australasia, during the 20th century has been well documented [29]. They are associated with both small and large flocks, feeding in loose aggregates. The birds feed on insects, earthworms, spiders, frogs and most especially ticks present on cattle and other large herbivores [16, 20, 32].

Generally, cattle egrets are colonial in nature and whitish. Many populations of cattle egrets are highly migratory and dispersive and this has helped the species' range expansion. The massive and rapid expansion of the cattle egret's range is due to its relationship with humans and their domesticated animals [48]. Both sexes of this bird are similar, with the male being slightly bigger and also possessing marginally longer breeding plumes than the female. The juvenile age group lacks coloured plumes and has a black beak [32], while the adult possesses greyish-yellow feet and sharply pointed short yellow bill [44]. The Western cattle egret is a medium sized bird, possessing short-legs and thick necks, in comparison to other egrets [20].

There is a dearth of literature on the documentation of the nervous system of the cattle egret. Literature search till date revealed published articles in this regard to include studies on the cattle egrets' cerebellum line drawing [23], Wulst volume [53], and more recently, the histology of the hippocampal complex [17] and craniofacial morphometrics [1]. Despite considerable advances toward the understanding of comparative avian neuroanatomy, there is hardly any literature yet on the comprehensive descriptions of the gross morphology of the cattle egret's brain and their neuromorphometric correlates, as well as their cerebellar histology. Hence, we here investigated the gross morphological features and morphometric parameters of the juvenile cattle egret's brain, as this is crucial to the exploration of the physical attributes of this species brain for easy identification and elucidation of some of its morpho-functional features, as well as consolidate the literatures on comparative avian neuroanatomy.

MATERIALS AND METHODS

Ethical considerations

Care was taken to ensure the birds experienced neither undue pain nor discomfort. The ethical clearance for the use of this bird was sought and granted by the Animal Care and Use Committee of the University of Jos, Nigeria (reference code: UJ/FPS/F17-00379). Also, the experimental protocols used were in conformity with the National Institute of Health Guide for the Care and Use of Animals (NIH Publications No. 80–23) and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Authors ensured that all ethical issues concerning plagiarism, approval to publish, errors in fabrication, double publication, and submission were adhered to.

Animals

The juvenile age group (n = 12, mixed sexes) of the captive and clinically healthy cattle egrets were sourced from Jos metropolis (GPS coordinates: 9°59'21.5"N; 8°52'49.7"E), a guinea savannah region of Nigeria [30]. They were aged according to the detailed description by [32] and [44]. The birds were procured alive at their habitat and immediately transported to the Gross Anatomy Laboratory of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Nigeria.

Perfusion and brain excision

The birds were sedated by the administration of ketamine (40 $mg.kg^{-1}$) and xylazine (10 $mg.kg^{-1}$) through the intravenous route, as described by [28]. The animals were

perfused transcardially in compliance with the protocol of [3] using 0.9% saline solution (for initial blood rinsing), followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB) solution at pH 7.4. The brains were carefully removed from the skull, and post-fixed for 48 hrs in 4% paraformaldehyde in 0.1M PB.

Gross morphological description and morphometrics

The gross anatomical observations were made on the brain in both *in situ* and *ex situ* positions. Gross images were captured using a Sony® digital camera (DSC-H300 20.1 megapixels with 35× Optical Zoom). The live bird weight was obtained using the mechanical laboratory weighing scale sensitive to 0.1 gm (Camry Emperor table scale J1712420834, China), while brain weight was obtained using the analytical weighing balance (Ohaus GmbH, Nänikon, Switzerland), sensitive to 0.01 gram, following its careful dissection from the cranium (*ex situ*). Linear gross morphometric parameters (Table 1) were measured with the aid of vernier caliper. The nomenclature here adopted in this study for the anatomical descriptions was as described in [38].

Schematic brain illustrations

The sketch of the cattle egret brain (for Figures 1A-C and 2Di, Ei and Ii) was made using Adobe Illustrator software (Adobe Inc., 2022. Adobe Illustrator version 27.0; available at https://adobe.com/products/illustrator). The pen tool was used to trace out the sketches from the bitmap image, to create a vector diagram. The images were imported into the artboard created, the pen tool was then used to trace out the edges of the image and also the various parts were traced out. Each of the different parts were then given different colours using the selection and colour swatch tools.

Definitions of measured gross parameters

The gross anatomical structures measured are concisely stated and defined as follows:

- Weight of the animal (WOA) (g): live bird weight to the nearest gram
- Weight of the brain (BOW) (g): weight of the whole brain without the meninges

Table 1. Mean ± SD values of measured parameters of the brain of the juvenile cattle egret

Measured parameters	Mean ± SD	No. of animals (n)
Body weight (WOB) [g]	538.33 ± 78	12
Brain weight (BRW) [g]	2.45 ± 0.34	12
Length of brain (LOB) [mm]	25.80 ± 1.98	12
Depth of brain (DOB) [mm]	16.86 ± 1.20	12
Length of cerebrum (LOC) [mm])	16.78 ± 1.15	12
Height of cerebrum (HOC) [mm]	10.83 ± 0.91	12
Width of cerebrum (WOC) [mm]	21.46 ± 1.42	12
Height of cerebellum (HCB) [mm]	8.97 ± 1.29	12
Length of cerebellum (LCB) [mm]	12.11 ± 0.68	12
Optic lobe length (OLL) [mm]	9.52 ± 0.49	12
Optic lobe width (OLW) [mm]	5.61 ± 0.32	12
Olfactory bulb length (OBL) [mm]	3.37 ± 0.56	12
Olfactory bulb width (OBW) [mm]	2.26 ± 0.19	12

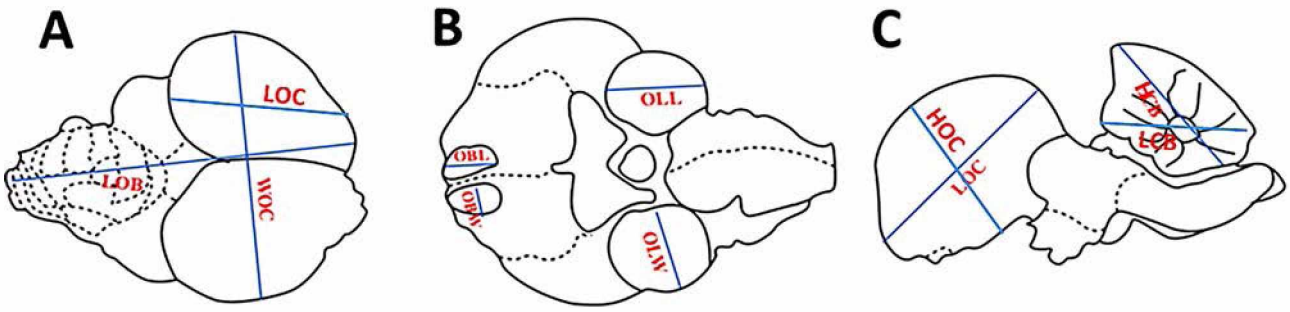

Fig. 1. Schematic illustrations of the measurements of major subdivisions of the brain of the juvenile Cattle egret (*Bubulcus ibis*)
Some of the measured gross parameters are depicted:
A – dorsal view: LOB: length of brain; WOC: width of cerebrum; LOC: length of cerebrum; **B** – ventral view: OBL: length of left olfactory bulb; OBW: width of left olfactory bulb; OLL: length of optic lobe; OLW: width of optic lobe; **C** – mid-sagittal view: LOC: length of cerebrum; LCB: length of cerebellum; HOC: height of cerebrum; HCB: height of cerebellum

- Length of brain (LOB) (mm): the maximum length from the tip of the olfactory bulb to the caudal most portion of the cerebellar cortex
- Depth of brain (DOB) (mm): the distance from the highest point of the brain (i.e., the topmost part of the cerebellum) to the lowest point of the brain (i.e., the most ventral part of the cerebrum/optic lobe)
- Length of cerebrum (LOC) (mm): the maximum length from the tip of the frontal lobe to the caudal most portion of the occipital lobe of the cerebral cortex
- Height of cerebrum (HOC) (mm): maximum dorso-ventral length of the cerebral cortex
- Height of cerebellum (HCB) (mm): maximum dorso-ventral length of the cerebellar cortex
- Length of cerebellum (LCB) (mm): maximum rostro-caudal length of the lobes of the cerebellum
- Width of cerebrum (WOC) (mm): maximum length across the most lateral portion of the parietal lobes of the cerebral cortex
- Optic lobe length (OLL) (mm): maximum length of the lobes of the optic tectum
- Optic lobe width (OLW) (mm): maximum width of the lobes of the optic tectum
- Olfactory bulb length (OBL) (mm): length of the (right or left) olfactory bulb from the tip (most rostral part) of the bulb to its caudal-most part
- Olfactory bulb width (OBW) (mm): maximum length across the latero-medial axis of the (right or left) olfactory bulb.

Histological processing of cerebellum

Immediately after the birds were sacrificed, the cerebelli were removed and fixed in 4% phosphate-buffered paraformaldehyde (Loba Chemie PVT Ltd, India). The tissue samples were processed using the routine histological technique as described by [2]. Briefly, the samples were dehydrated in graded alcohol concentrations, cleared in xylene and infiltrated and paraffin wax embedded. Surgifield SM-202A rotatory microtome was used for the sectioning at 5 µm. The processed sections were stained using Haematoxylin (CDH lab reagent, New Delhi, India) and Eosin (Kem Light lab reagent, Mumbai, India). Photomicrographs were taken at ×40, 100 and ×400 magnifications through an Olympus microscope equipped with industrial digital camera using the imaging software, MII ImageView version 3.7.9229 (YSC Technologies, Fremont, CA, USA). Histological examination was carried out on the processed tissue samples and photomicrographs of the slides were taken.

Data analysis

The numeric raw data generated from the brain gross morphometry were analysed and expressed as mean ± SD, and the Pearson correlation coefficient (R two-tailed test; $P<0.05$) was used to compare the means and the strength between the variables and their relationships. The GraphPad Prism software (GraphPad Prism version 7 for Windows, GraphPad Software, San Diego, California USA), and the JMP SAS/STATR software version 10.0 (SAS Institute Inc., Carry, NC, USA) were used for all statistical analyses.

RESULTS

Figures 1–2 reveal the various anatomical regions of the brain of *Bubulcus ibis* studied with detailed labelling of measured parameters. The data obtained for the mean ± SD values of the measured parameters of the brain of the juvenile cattle egret and their correlation analyses were shown in Tables 1 and 2, respectively. The low and high-power photomicrographs of the H&E-stained slides of cerebellum were shown in Figure 3. The descriptive terms here adopted for the gross brain features are in accordance with [26, 38], while that of the histological features were as described by [47] and [23].

Brain gross morphology

Figures 2A-I illustrates the different anatomical regions of the brains of the cattle egrets' studied. The brain was entirely located in the cranium (Figure 2A). The brain was entirely covered and tightly packed by meninges which was a tough fibrous sheath with a whitish colouration (Figure 2B). The *Bubulcus ibis* brain basically consists of the similar components with that of mammals and regionally, it is organized into: the forebrain (prosencephalon), mid-brain (mesencephalon) and the hindbrain (rhombencephalon).

For easy description, the brain of the juvenile cattle egret in this study was divided rostro-caudally and regionally into olfactory bulb, the cerebrum, the optic lobe (lobus opticus), the cerebellum and the pons and medulla oblongata (Figures 2Di, Ei and Ii), with the various anatomical

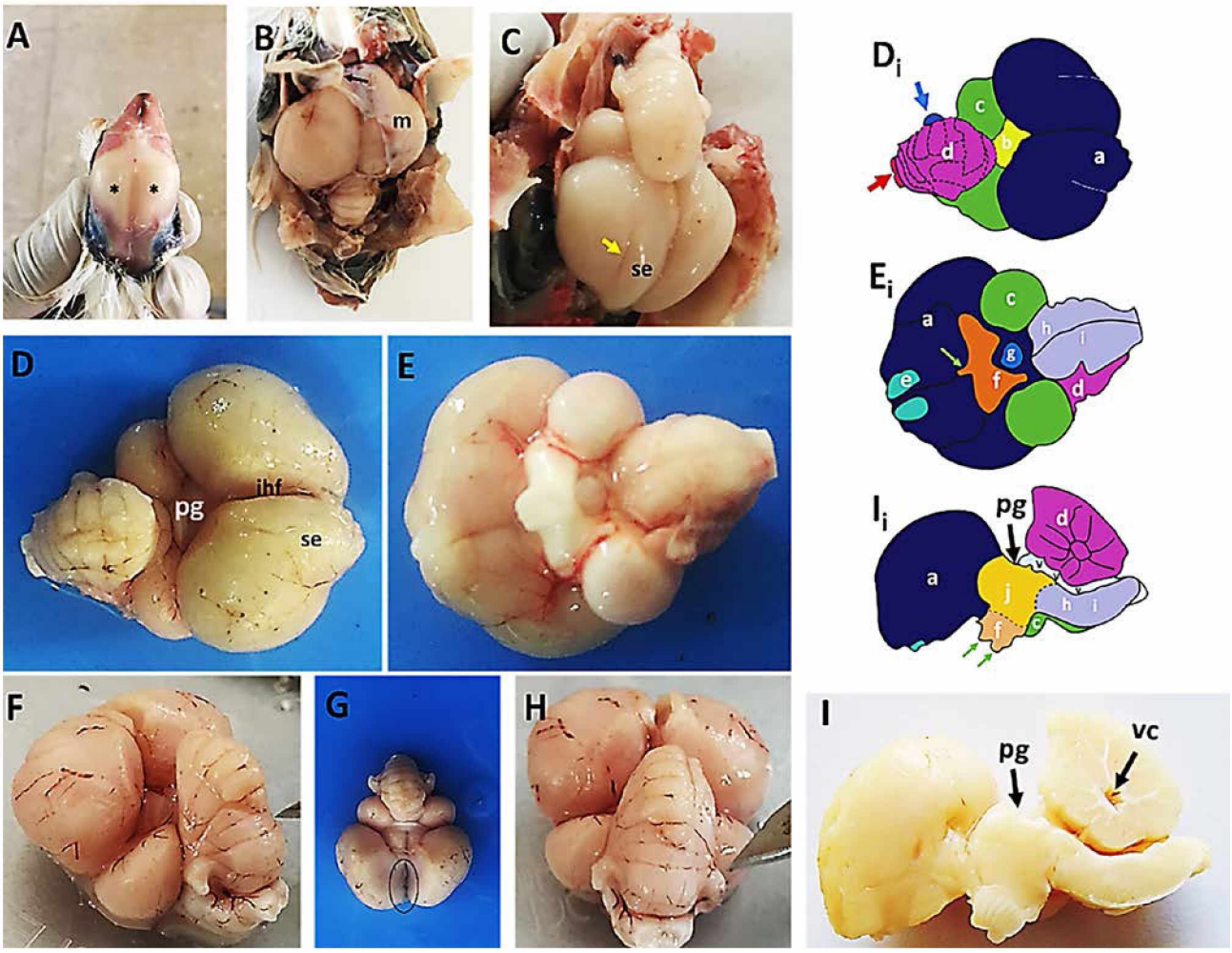

Fig. 2. Brain of the juvenile cattle egret (*Bubulcus ibis*) *in situ* and *ex-situ*

A–shows the cranial covering of the unexposed brain. Black asterisks indicate bony cranium; **B**–following the complete removal of the cranium, the portion partly covered with meninges and the exposed portion of the brain are shown. Black arrow shows the reflected part of the meninges while m is unreflected meningeal covering; **C**–show the dorsal view of the brain with the ventral part still situated in the skull (*in situ* position), with prominent appearance of "three superstructures" viz cerebrum, optic lobe and cerebellum. sagittal eminence (se), yellow arrow points to vallecula telencephalic; **D** and **G**–dorsal view, *ex situ*. Black circle placed at the interhemispheric fissure (ihc) in G indicates absence of the corpus callosum. Di illustrates prominent structures such as the cerebral cortex (a), pineal gland (b), optic tectum (c), cerebellum (d), while the blue arrow points to the auricula cerebelli and the broken white lines on the rostral part of cerebrum indicates the vallecula telencephali. Red arrow refers to the initial segment of the spinal cord. **E**–ventral view of the brain, Ei illustrates the olfactory bulb (e), optic chiasm (f), hypothalamic area (g), pons (h) and the medulla oblongata (i), while the green arrow points to the projection of the optic nerve; **F**–lateral view; **H**–caudal view; **I** and Ii mid-sagittal views – prominently show the diencephalon. Ventriculus cerebelli (vc); pineal gland (pg) (j), and the 4th ventricle (v)

Note: Di, Ei and Ii are reconstructed images

regions characterized by their own peculiarities. The anatomical characteristics of the different parts of the cattle egrets' brain are listed below:

Olfactory bulbs

Olfactory bulbs (*Bulbus olfactorius*) were very reduced and tapered to the rostro-ventral pole of the cerebral cortex. The bulbs were coplanar and conical-like in shape (Figure 2E). The olfactory bulbs were barely visible when the brain was viewed from a dorsal position (Figure 2D) but prominent in the ventral view (Figure 2E). Grossly, there was no discernible division of the olfactory brain into the olfactory bulb, the olfactory tract and the olfactory lobe. The olfactory bulb is paired and projected from the two hemispheres of the cerebrum, the two small bulges connected together by the indistinct olfactory tract. The olfactory bulb of the cattle egret assumed the same colouration as the entire brain structures, and rhinal fissure (which was a small depression) separated the olfactory bulbs from cerebral hemispheres.

Table 2. Pearson correlation coefficient (R two-tailed test) of means and strength (variables vs relationships) of measured parameters of the brain of the juvenile cattle egret

	BOW	BRW	LOB	DOB	LOC	HOC	WOC	HCB	LCB	OLL	OLW	OBL	OBW
BOW	–	0.92	0.77	0.33	0.27	0.45	0.55	0.44	0.81	0.45	0.59	0.33	-0.01
BRW	0.92	–	0.85	0.54	0.49	0.68	0.76	0.64	0.75	0.60	0.74	0.60	0.13
LOB	0.77	0.85	–	0.55	0.62	0.49	0.67	0.69	0.79	0.37	0.65	0.66	0.21
DOB	0.33	0.54	0.55	–	0.57	0.43	0.61	0.43	0.27	0.54	0.39	0.45	0.66
LOC	0.27	0.49	0.62	0.57	–	0.40	0.47	0.44	0.38	0.32	0.34	0.51	0.57
HOC	0.45	0.68	0.49	0.43	0.40	–	0.68	0.58	0.41	0.76	0.71	0.67	0.30
WOC	0.55	0.76	0.67	0.61	0.47	0.68	–	0.84	0.39	0.59	0.81	0.70	0.23
HCB	0.44	0.64	0.69	0.43	0.44	0.58	0.84	–	0.34	0.21	0.83	0.90	0.25
LCB	0.81	0.75	0.79	0.27	0.38	0.41	0.39	0.34	–	0.47	0.44	0.26	0.04
OLL	0.45	0.60	0.37	0.54	0.32	0.76	0.59	0.21	0.47	–	0.46	0.21	0.26
OLW	0.59	0.74	0.65	0.39	0.34	0.71	0.81	0.83	0.44	0.46	–	0.81	0.16
LOH	0.01	0.16	0.21	0.45	-0.04	0.24	0.23	0.44	0.15	0.05	0.29	0.44	0.25
OBL	0.33	0.60	0.66	0.45	0.51	0.67	0.70	0.90	0.26	0.21	0.81	–	0.24
OBW	-0.01	0.13	0.21	0.66	0.57	0.30	0.23	0.25	0.04	0.26	0.16	0.24	–
WHO	0.36	0.48	0.55	0.35	0.43	0.28	0.43	0.74	0.27	-0.11	0.53	0.76	0.30

Cerebral hemispheres

The cattle egret brain is lissencephalic, with the cerebral cortex (*pallium*) smooth-surfaced and lacking a discernable gyri and sulci. From a dorsal view, the shape of the cerebrum assumes that of an obtuse triangle, there was a hemicycle-like expansion of the left and right lateral parts, and a wide posterior part in contact with the most rostral portion of the optic lobe (Figures 2D, F, H). The external surface appeared smooth and the corpus callosum was conspicuously absent (Figure 2G). A prominent interhemispheric fissure separates the two cerebral hemispheres (Figure 2D). Each hemisphere presented a shallow groove with a caudo-lateral orientation, this groove is known as the *vallecula telencephalic* which has an appearance of a cambered super-sulcus, and was richly engrossed by large blood vessels that caudally tappers out (Figure 2Di). Between the interhemispheric fissure and the vallecula is an ellipse-shaped bilateral protuberance known as the sagittal eminence (*eminentia sagittalis*), and this appeared slightly elevated above the cerebral cortical portion lateral to it (Figures 2C-D, Di).

There was a prominent indention (due to their relatively large eyes of this bird) on the lateral aspects of the cerebral hemispheres known as the *fovea limbica*. The medial surface of the cerebrum was smooth and straight (Figure 2I). A transverse sulcus (*Fissura subhemispherica*) was present between the most caudal part of the cerebral hemispheres and the rostral part of the cerebellum it borders (Figures 2D-H).

Diencephalon

The diencephalic structures were located on the ventral aspect of the cerebral hemispheres and were completely covered by telencephalon. They were almost invisible in the dorsal and ventral views (Figures 2D-E) of the brain but prominent in the mid-sagittal/median section of the brain (Figure 2I). The diencephalic structures represent the rostral limit of the brain stem and they were completely covered by the cerebral hemispheres (*hemispherium cerebri*). The pineal gland is a small structure occupying the polygonal space at the junction of the transverse sulcus, cerebral hemispheres, optic lobe and the cerebellum (Figures 2D, G). The diencephalon was bilaterally linked with the mesencephalic tectum, with the pineal gland appearing to form its dorsal limit (Figures 2D, G, I).

Mesencephalic tectum

The mesencephalon of the cattle egret brain is chiefly constituted by the mesencephalic tectum (optic lobe), a monologue of the mammalian anterior colliculi. The mesencephalic tectum formed the bulk of the roof of the midbrain. The optic lobes were large, paired oval eminences protruding laterally and ventrally from the surface of the midbrain, and lying lateral to the more ventral tegmentum (Figures 2D-H). The midbrain bordered the rostral portions of the rhombencephalon (cerebellum and pons), with a transverse furrow delineating the boundary between the mesencephalon and the rostral rhombencephalon. The optic lobes receive the fibers (optic tract) of the optic chiasma at its convergence in the ventro-median line (Figure 2E). No obvious demarcation exists between the midbrain, pons and the medulla oblongata.

Cerebellum

The metencephalon comprises the pons and cerebellum, while the myelencephalon is constituted by the medulla oblongata. The cerebellum of the cattle egret encloses the fourth ventricle (*ventriculus quartus*) and forms its lateral walls and roof (Figure 2I).

The cerebellar cortex lies over the pons, medulla oblongata, and the median segment of the mesencephalon (Figures 2F-H). It is connected bilaterally to the brainstem by rostral and caudal cerebellar peduncles (one of these is evident in Figure 2I). The superficial layer of the cerebellum (cerebellar cortex) is grey matter and it covers the internal white matter (cerebellar medulla). There was a centrally-placed apparent vermis and laterally positioned auricles. Multiple strips of superficial and deep transverse sulcus distributed on the cerebellar vermis divide the cerebellar vermis into lobules. The cerebellar auricles are the paired bilateral posterio-lateral extensions of the cerebellum and were located at the ventrolateral surface of the cerebellar vermis (Figures 2D, F, H). Dorsally, there was an extension of the fourth ventricle into the cerebellum and this is known as the *ventriculus cerebelli* (vc) (Figure 2I).

Pons and medulla oblongata

The pons and medulla oblongata were located on the ventral surface of the cerebellum. They are the two posterior parts of the brain stem that extend from the most caudal part of the midbrain to the occipital foramen. A ventral middle fissure (a longitudinal supersulcus), the *fissura mediana ventralis* runs its course medially through the pons and the medulla oblongata (Figures 2E, Ei). The dorsal surface of the pons, jointly with the medulla oblongata, form the ventral wall for the fourth ventricle, with their ventral surface forming the lowest spot of the brain stem (Figures 2E, I). there appears to be no discernible borders delineating the boundaries of the midbrain, pons and medulla oblongata.

Brain weight and gross morphometry

Figure 1 illustrates the landmarks for the gross morphometric parameters taken and the results of the morphometric measurements were presented in Table 1 and the correlations analyses of the parameters were presented in Table 2.

The average recorded body weight was 538.33±78 g, while the brain weight was 2.45±0.34 g, implying that the brain constituted about 0.46% of the total body mass (Table 1). The cerebral cortex length makes the most of the brain length with a contribution of about 52%, followed by the cerebellum (about 38%) and olfactory bulb (about 10%) to the total brain length value, as shown in Table 1. The optic lobe of the cattle egret was about as twice longer than its wide, with the olfactory bulb length and width values sharing similarity in values.

The body weight was strongly positively correlated with the brain weight ($r=0.92$; $P<0.001$) and the length of the brain ($r=0.77$; $P=0.001$). As shown in Table 2, the brain weight was strongly positively correlated with the length of the brain ($r=0.85$; $P<0.001$), width of cerebrum ($r=0.76$; $P=0.004$) and the length of cerebellum ($r=0.75$; $P=0.005$). Also, the olfactory bulb length was strongly positively correlated with the height of cerebellum ($r=0.90$; $P<0.001$), and width of the optic lobe ($r=0.81$; $P=0.002$).

Cerebellar histology

The histological appearance of the cerebellum (Figures 3 A-B) was similar in all the examined animals. The cerebellar cortex had the three typical cerebellar layers (Figures 3 C-F) and there was the innermost cerebellar medulla (Figures 3 C-D). The molecular cell layer was the outermost layer and it is composed of small neurons and neuroglia. The Purkinje cell layer was sandwiched between the outer molecular and inner granular layers, and it presented a single row of predominantly flask/pear-shaped

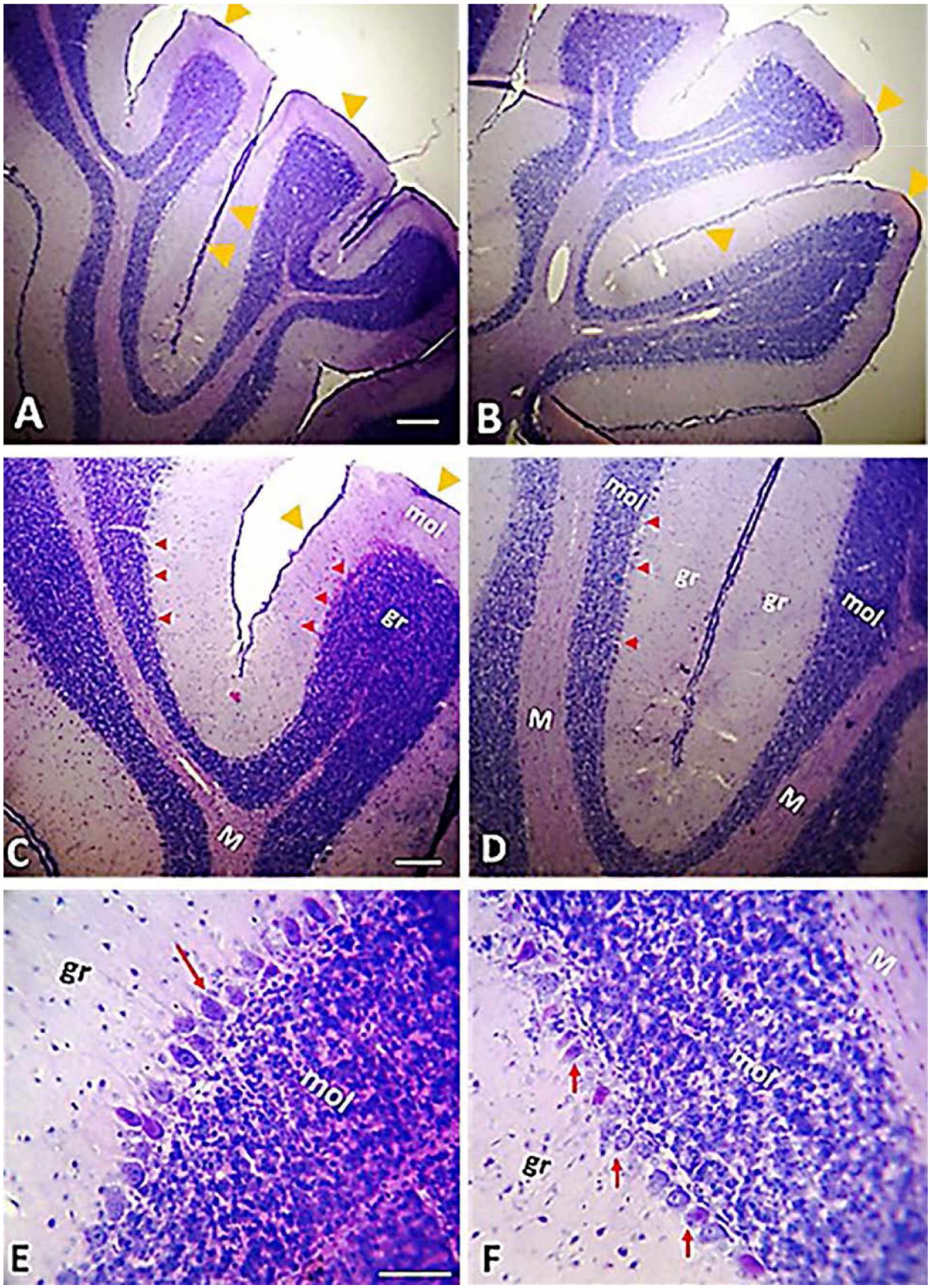

Fig. 3. Juvenile cattle egret cerebellum

A–D – low power photomicrographs. E–F – high power photomicrographs. External granular layer (yellow arrow heads), molecular layer (mol), inner granular layer (gr), Purkinje cell layer (red arrow heads), M – medulla, Purkinje cells (red arrows) Scale bars: A–B, 150 μm; C–D, 100 μm; E–F, 50 μm

cells and some round/oval-shaped cells, showing distinct nuclear materials and nucleoli, and bearing prominent dendrites with threadlike appearances (Figures 3 E-F). Of note, there were traces of persistent external granular layer, as evident in Figures 3 A-C. The inner granular layer presented numerous round granule cells, and the cerebellar white matter possessed sparse granule neurons enriched with cellular processes.

DISCUSSION

We here described the gross anatomical features and gross morphometry of the juvenile cattle egret (*Bubulcus ibis*) brain as well as the cerebellar histology. The present study showed that the brain of the cattle egret consists of prosencephalon, mesencephalon and the rhombencephalon and this conforms to the structural pattern of brain morphology in avians [26].

Grossly, the olfactory bulbs of the cattle egret were rudimentary and poorly developed (relatively to the other structures) with no discernible division of the olfactory brain into the olfactory bulb, the olfactory tract and the olfactory lobe, with the two small bulges connected together by the indistinct olfactory tract and were barely visible from a dorsal position but prominent in the ventral view. This shares similarity with the observation of rudimentary olfactory bulbs in Sparrowhawk (*Accipiter nisus*), Coturnix quail and domestic birds [4, 12, 31, 37], however, some diurnal birds such as the wild African parrot, canaries and vultures [36, 51], as well as nocturnal birds' species such as the Kiwi and Barn owl reportedly have well developed olfactory bulbs [31]. In most avian species, previous evidences suggested that structural parts associated with sense of smell were clearly atrophied and poorly developed relatively to some other species besides avians. Comparatively, the observation by Usende et al., [50] in the reptilian African side necked turtle (*Pelusios castaneus*), revealed that the olfactory brain was elaborate, and presented an initial olfactory bulb, a distinct middle olfactory tract and a caudal olfactory lobe. This may not be unusual, as well developed and conspicuously evident olfactory brains in lateral, ventral and dorsal views are typical of reptiles, rodents and Siluriformes brains [35, 49, 54]. However, in similarity, completely inconspicuous and dorsally invisible olfactory bulbs have been reported in man and elephant [46].

Also, we reported herein that the cerebral hemispheres of the cattle egret appeared smooth, lacking gyri and sulci, a phenomenon known as lissencephaly. Generally, the avian brains are regarded as lissencephalic. Smooth-surfaced brains have been reported in birds such as the Coturnix, African ostrich, Sparrowhawk and domestic birds [4, 10, 12, 37, 43] in accordance with our findings. Of note, similar lissencephalic brains have also been reported in some rodents such as the African giant and grasscutter rats, reptiles, and sea turtles [8, 19, 39, 54].

We observed the prominent bulge of the sagittal eminence and obvious vallecula telencephali on the dorsal surface of the cerebral hemispheres, which conforms to the observations in the wild African Parrot [51] and Macaw [34], Common starling [10], African ostrich [43], Emu (a diurnal, flightless) and Barn owl (a nocturnal flying bird), but those features were reportedly less pronounced in the various breeds of chicken [5, 13, 14, 42], pigeon (a diurnal flying bird) and non-prominent in the Kiwi, a nocturnal flightless bird [31]. The vallecula in avian supports visual acuity, and its prominence could enhance the visualization of prey, especially in wild birds [51].

The cattle egret possessed a well-developed optic lobe which bulged laterally with prominent optic tracts appearing as large bands of the white matter on the ventro-medial parts of the optic lobes. The optic tracts crossed just cranial to the hypothalamic portion of the diencephalon bearing a connection with hypophysis to form the optic chiasma. This clearly conforms to the observations of grossly developed and highly visible mesencephalic tectum in the Sparrowhawk [4], Coturnix quail [12], African ostrich [25, 43], domestic birds [37] and the pigeon [18, 31] and helmeted Guinea fowl [52], but differs from the reportedly less developed optic lobes of the Barn owl, Kiwi, Humming birds and wild African parrot [31, 51]. Birds are strongly visual animals as a result of the extensive development of nuclei and pathways concerned with processing of visual signalling in the CNS [26]. This likely explains the large mesencephalic tectum (optic lobe) of the cattle egret which appeared as paired oval eminences forming the bulk of the midbrain.

It's been reported that morphometric analyses of organs may elucidate minute structural changes that may not be evident in ordinary qualitative analyses, and quantitative assessments have been used to unveil the morphologic and functional capabilities of brain regions in different animals [40]. Since the beginning of the 19th century,

documentations of the morphometric studies on various organs of avian species started and reports on it has been on the increase. So far in the last two decades, several comparative studies have been reported in which different variables such as the ecological and social attributes have been deployed to expound the variabilities in vertebrate species' brain sizes [47]. Also, environmental factors like an animal's habitat, lifestyle, and their morphological and physiological parameters, have been correlated to their evolutionary history [6, 43].

In this study, the average recorded body weight of the cattle egret was 538.33±78 g, this is in line with the weight range (270–512 g) of the cattle egret as documented by Telfair and Raymond [48]. Our body weight data strongly positively correlated with the brain weight ($r=0.92$; $P<0.001$). The average length of brain here recorded for the cattle egret was 25.80±1.98 mm, this value was similar to that of Sparrowhawk (24.3 mm) but higher than that of Coturnix quail brain (16 mm) [4, 12]. Significant increase in the length and width of the cerebral hemispheres with advancing age has been reported in the chicken [13, 42]. Interestingly, Peng et al. [43] and Karkoura et al. [25] reported higher values in the average mean length and width of optic lobes of adult African ostrich than we recorded in our study on the juvenile cattle egret. Wanmi et al. [52] reported that the mean weight of the midbrain of the helmeted Guinea fowl increased with advancing in age, hence the variability in morphometry of optic lobe and brain length might be due to age and/or species differences. Overall, our regression analysis data ($r^2=0.98$) indicates that the weight of the animal can be excellently predicted from parameters such as the brain length, cerebral width, cerebellar height, optic lobe width and the olfactory bulb length (data not shown).

The developmental form in various avian species has been reported to correlate with the differences in their relative brain volume [21]. The average brain weight of the cattle egret used in this study was 2.45±0.34 g, this was slightly lower than average weight (3.00±0.2 g) of the Sparrowhawk brain [4] and much lesser than that of the domestic fowl (3.34±0.08 g) [15]. The juvenile cattle egret brain weight constituted about 0.46% of the total body mass. This is in contrast to reports in some avian species, where the relative brain to body mass index was reported to be 0.015% in the African ostrich, 0.26% for the oriental white stork, 0.26% in the Pekin duck, and the grey goose brain accounted for 0.25% of the total body weight [43]. The above report suggests that the encephalic ratio of the cattle egret is the highest compared to the other listed birds. This could be due to the age categories of birds under consideration as we used juvenile age group in our study, whereas the adult age bracket was considered for the African ostrich study with the age groups for the other birds undisclosed by their authors. Also, relatively larger brains could be associated with the length or prolongation of the nestling stage of birds [21].

The gross anatomical organisation and histological layering of the cerebellum is similar in most vertebrates, though some differences have been reported according to species' general morphology and behavioural attributes [22, 43, 47]. Iwaniuk et al. [23] reported on the cerebellum of cattle egret using a line drawing. The authors showed that the cattle egret cerebellar folia organization followed the "typical" avian cerebellar morphology, with a presentation of eleven major folia. The result of our study on the juvenile age group was in conformity with the above report, and were have also shown the cellular organization of the cerebellar layer of this bird by the use of the H&E stain. Interestingly, the cerebellar histology of the juvenile cattle egret we here studied presented a persistent external granular layering suggestive of a potential for adult neurogenesis. The cerebellum is widely used in studies on motor system, this is because it is responsible for the maintenance of equilibrium and motor disorders are mostly associated with cerebellar dysfunctions. The cellular components of the cerebellum function as a control system located between motor and cerebellar dysfunctions pathways [41]. Our gross description of the cattle egret cerebellum aligned with the reports on that of the African ostrich, Sparrowhawk and domestic birds [25, 37, 43], but in contrast with the small, rounded/oval and unfolded cerebellum of the wild African parrot [51]. Of note, most large-sized birds (especially flightless or short distance flyers) reportedly possess relatively small-size of cerebellum [24].

The most emphasized and studied cells of the cerebellum in behavioural and cognitive studies are the Purkinje cells, especially with respect to their functional capability. A comparative study in the pigeon, turkey, ducks and starling revealed that increasing number of Purkinje cell resulted in an increase in behavioural complexity and cognitive ability [47]. The presence of complicated pleats in

the superficial grey matter of the cerebellum has been attributed to its' larger surface area. In the African ostrich, the cerebellum protrudes visibly upward and its length was reported to be about 1.5 times larger than its height [43]. These findings conform to our observation in the cattle egret, with cerebellum elevated above the cerebral hemispheres and the cerebellar length 1.4 times larger than the cerebellar height. Our histological profiling of the cattle egret cerebellum has added further data and relevant literature to comparative avian cerebellar histology.

CONCLUSIONS

The morphological, morphometric and histological studies on the cattle egret brain are very important for comparative and developmental studies and evolutionary neuroscience, as the research on cattle egret brain morphology during the different developmental phases is lacking in the literature. Hence, we believe that the macro-anatomical structures and histology of cerebellum of cattle egret here described has provided supplementary data to the basic information on the Avian CNS. However, further studies are required especially on the aging-associated morphological and morphometric indices, as studies across age groups are of great value to set standards of comparison for research. Also, the detailed description of the internal structure of the cerebrum as well as the embryonic development/foliation levels of the cerebellum in this species will be described in further studies. Our future research directions also entail the neurocellular and neurochemical profiling of the distinct cells in various regions of the cattle egret brain.

DECLARATION OF COMPETING INTEREST

All authors declare no conflicts of interest.

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