Gross morpho-biometry, histometry and immunohistochemical investigations of pituitary gland in the juvenile and adult male African giant rats (*Cricetomys gambianus*)

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SUMMARY

This work was designed to investigate the morphometry and immunohistochemical features of pituitary glands in the African Giant Rats (AGR). Adult and juvenile AGR were sacrificed and their brains harvested, and the pituitary glands were carefully dissected. The weight, length and width of brains and pituitary glands were subsequently measured. Sections were prepared and stained using Haematoxylin and Eosin (H&E), Cresyl-violet and periodic-acid-Schiff (PAS) for histological analysis. Immunohistochemical analysis was carried out with Glial-Fibrillary-Acidic-Protein (GFAP) and Ionized-Calcium-Binding-Adapter-Molecule 1 (Iba1). Grossly, the AGR pituitary gland is a somehow laterally extended, saddle-shaped organ that is dorso-ventrally flattened. The gray-coloured adenohypophysis (anterior-pituitary lobe) was bigger in size than the whitish neurohypophysis (posterior-pituitary lobe) on physical examination. Histometrically, the adenohypophyseal length was noticeably greater than that of neurohypophysis in both juvenile and adult. Similarly, the adenohypophyseal and neurohypophyseal width in adult were found to be more than that of juvenile. Also, the histological staining of the neurohypophysis and adenohypophysis were distinct. In the neurohypophysis, fibrous and protoplasmic pituicytes were observed among fibres and Herring-bodies. Chromophils and chromophobes were identified in the adenohypophysis and melanotrophs in the intermediate lobe. Immunohistochemistry showed pituicytes in the neurohypophysis, which was positive with GFAP-antibody. With Iba1-antibody, the neurohypophysis expressed a stronger positive immunolabelling to microglia as compared to macrophages in the adenohypophysis. In conclusion, the gross and microscopic characteristics of pituitary glands of the AGR are found to be similar to other rodents and mammals. We recommend further study to compare morphometrical parameters between male and females of this model.

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INTRODUCTION

The African Giant Rats (AGRs) (Cricetomys gambianus) are nocturnal rodents, feeding omnivorously both on vegetation and invertebrates. They inhabit a variety of habitats ranging from arid to temperate regions, but need some form of shelter to survive (Ajayi, 1974; Nzalak et al., 2005). The size of AGR makes them suitable for broader range of experiments and studies and they can live longer in captivity, thereby providing an extended time window for longitudinal studies. The most significant and interesting attribute of AGR in research is their exceptional sense of smell. They have a highly developed olfactory system, which enables them to detect and distinguish a wide range of scents (Olude et al., 2014; Freeman et al., 2020). This advantage has been utilized in detecting landmines and tuberculosis in field studies. The AGR has been proposed as an adaptable native research model due to its unique neuro-behavioural attributes, and thus the need to establish baseline information on its nervous system (Mustapha et al., 2019).

The pituitary gland is a hypothalamic structure of the brain and serves as a master gland that controls almost all the activities of other glands in the body. The pituitary gland is divided into adenohypophysis (anterior-pituitary lobe) and neurohypophysis (posterior-pituitary lobe); it is situated within the hypophyseal fossa of the sphenoid bone called sella turcica, and it is covered by diaphragma sellae. Studies have claimed the variation in size of the pituitary gland associated with age, gender and pathological conditions (Lamichhane et al., 2015). Alteration in the shape and size of the pituitary gland can result in abnormal nervous processes and hormonal behaviours (Emerald, 2016). There are many reported anatomical investigations on the pituitary glands of mammals, including the greater cane rat (Gilbert et al., 2020), the rabbit (Vionna et al., 2020), the guinea pig (Amat, 1970; Luay, 2016), the rat (Mixner and Turner, 1942; Montemurro, 1964; Popoola and Sakpa, 2018), the mink (Weman and Nobin, 1973; Weman, 1974; Cardin et al., 2000), the hedgehog (Treier et al., 2001; Botermann et al., 2021) and the viscacha (Acosta and Mohamed, 2009); however, the pituitary gland of AGRs has been rarely investigated in detail, especially anatomical investigation. AGR being an indigenous rodent, it will be then interesting to look at the pituitary gland and generate baseline anatomical data, since there is an increasing demand for indigenous animal models for scientific research. The measurements of selected parameters of the brain excluding pituitary gland of the AGR have been documented by Olude et al. (2016) and Nzalak et al. (2005). The present work, therefore, was designed to investigate the gross biometric and histometric features in both adult and juvenile male AGR, and to determine the basic cellular architecture and immunohistochemical expression of the pituitary glands in the AGR in order to provide basic research data, which will add to the understanding of the biology in this unique African rodent.

MATERIALS AND METHODS

Ethics

The animal experiment was approved by Animal Care Committee of National Veterinary Research Institute, Vom (NVRI/AEC/03/11622).

Animals

A total of 15 male AGRs were used for this study. The rats were divided based on age gap into two groups of nine (9) adults and six (6) juveniles. Age groups were estimated in accordance to Ajayi (1974) (Juveniles: >70g but <500g; Adult: >500g). They were sourced from Oranyan Market and University of Ibadan in Ibadan metropolis, Oyo State, Nigeria. They were transported live in cages to the experimental animal house, Neuroscience Unit, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The rats were allowed to acclimatize for a few hours prior to sacrifice. At the time of sacrifice, the body weight of each rodent was obtained with a digital electronic balance (Zhongshan Camry Electronic Co. Ltd, China).

Extraction of Brain and Pituitary Gland

The rats were sacrificed after the protocol described by Olopade et al. (2011). They were deeply anesthetized intraperitoneally (ketamine 100 mg/kg body weight and xylazine 10 mg/kg body weight) and perfused transcardially with normal saline followed by 10% neutral buffered formalin (NBF). The brains were subsequently harvested from the skull, and the pituitary glands were carefully dissected. Both the brains and pituitary glands were examined *in situ* to rule out any pathology before removal for gross morphological investigation. Gross biometry of the pituitary gland was measured and described according to the method of Ju et al. (2010) and Rahman et al. (2011). The pituitary glands were trimmed of meninges, and their weight was measured using Electronic Balance FA2004B (Shanghai York Instrument Co. Ltd., China). The length (rostro-caudal distance) and width (distance between the lateral edges) of the glands were obtained using stainless steel Electronic Digital Caliper K-319 (Kales Tool Industry & Trade Co., Ltd., China), as indicated in Fig. 1. The recorded measurements were used to calculate Organo-somatic Index (OSI) of the brain and pituitary gland, by dividing organ weight with body weight multiplied by 100 (weight of organ (g)/ weight of body (kg) x 100), as described by Radhiah and Azhar (2020).

Tissue Processing for Light Microscopic Study

The pituitary gland tissues were processed for routine paraffin embedding. Five microns-thick sections were cut from the paraffinized tissue blocks unto glass slides, using a microtome (Microm GmbH, D-6900 Heidelberg, West Germany). The sections were deparaffinized and stained with Haematoxylin and Eosin (H&E) and Cresyl violet for general histological examination (Gilbert et al., 2022). Tissue sections were subsequently stained with Periodic acid-Schiff (PAS) special stain, as described by Kondoh et al. (2017). The stained tissue sections were visualized using a light microscope (Leica Microsystems, Wetzlar, Germany) equipped with a CEFC Industrial digital camera. Histometry was carried out using the Motic® Images Plus 2.0 ML software (Motic Asia, Kowloon, Hong Kong).

Table 1. The mean body, brain and pituitary gland weights.

Parameters / Group	Number of animals	Mean Body Weight (g) Mean ± SEM	Mean Absolute Brain Weight (g) Mean ± SEM	Mean Absolute Pituitary Weight (g) Mean ± SEM
Adult	9	984.8 ± 34.09	5.6 ± 0.18	0.019 ± 0.0021
Juvenile	6	493.7 ± 23.41	4.42 ± 0.11	0.012 ± 0.0054

SEM = standard error of mean; g = grams; Normality test (Shapiro-Wilk test) = body (adult: P<value 0.356; juvenile P<value 0.093), brain (adult: P<value 0.612; juvenile P<value 0.540), pituitary gland (adult: P<value 0.149; juvenile P<value 0.082)

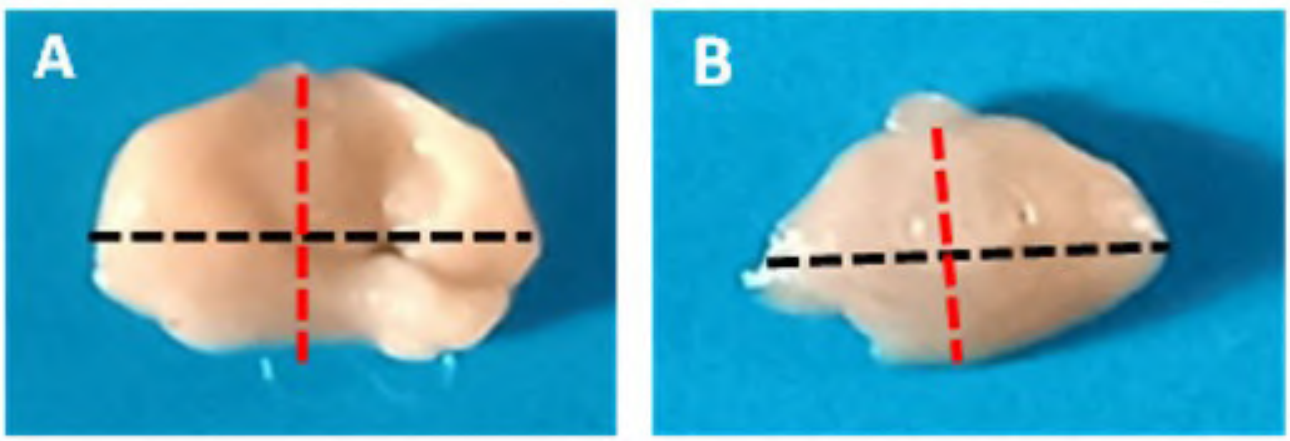

Fig. 1.- The pituitary gland of AGR. **A:** Showing the rostro-caudal (red dot-lines) and transverse distance (black dot-lines) from the dorsal view in adult AGR. **B:** Showing the rostro-caudal (red dot- lines) and transverse distance (black dot-lines) from the dorsal view in juvenile AGR.

Immunohistochemistry

The tissues were prepared for immunohistochemical examination based on the protocol described by Gilbert et al. (2020). Briefly, tissue sections of 5 µm thick paraffinized pituitary tissues were mounted on well-labelled charged glass slides and baked in an oven (60°C) for 90 minutes in order to melt the wax. The paraffinized tissue sections were subsequently deparaffinized in two changes of xylene, and then hydrated in descending concentration of alcohol to water. Retrieval of antigen was done with 10 mM citrate buffer (pH of 6.0) in order to unmask the hidden antigenic site. Tissue sections were treated with 30% H_2O_2/methanol so as to stop endogenous peroxidase activities and prevent non-specific binding by antibody. 2% PBS milk was used for blocking via incubation for 60 minutes and tissue sections were subsequently immune-labelled with these antibodies: rabbit anti-Iba1 antibody (dilution 1:1000, Wako Pure Chemical Industries Ltd., Japan) for microglial cells and rabbit anti-GFAP antibody (1:1000; Dako, Denmark), to visualize pituicytes or astrocytic-like cells. The primary antibodies were diluted in 1% PBS milk and 0.1% Triton X detergent and incubated overnight at 4°C. Secondary antibodies (dilution 1:200, Abcam Inc, USA) were subsequently added and the end product of entire reaction was improved with a chromogen, 3,3'-diaminobenzidine (DAB) (1:25 dilution, Vector Laboratories, USA) for 5 minutes. After dehydration of tissue sections in graded solutions of alcohol concentration, double changes of xylene were further used for 5 minutes each to remove the alcohol. Lastly, the tissue sections were mounted wet in permount, cover slipped and allowed to dry before microscopy (Leica Microsystems, Wetzlar, Germany).

Statistical analysis

Data are expressed as Means ± SEM and the differences among groups were considered significant at p-value < 0.05. Data were analysed using Excel software. Normal distribution of the data was confirmed with normality test (Shapiro-Wilk test), then unpaired t-tests and descriptive statistics were carried out using GraphPad Prism version 9.0.0 (GraphPad Software, San Diego).

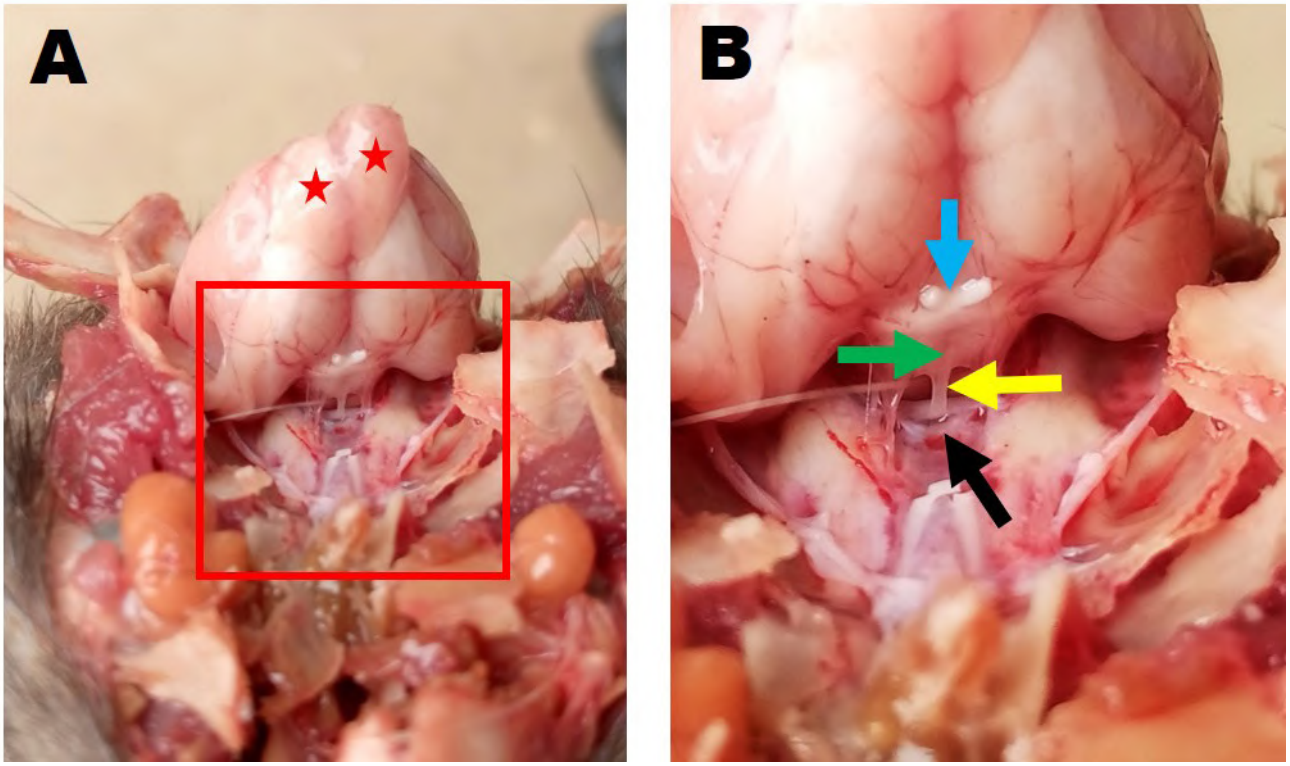

Fig. 2.- Photograph of head of the AGR showing the connection of pituitary gland to the base of brain. Lower (**A**) and higher (**B**) magnification of the head showing the pituitary gland (black arrow) still connected to the median eminence (green arrow) through the pituitary stalk (yellow arrow). Blue arrow = optic chiasma, red stars = olfactory bulb.

RESULTS

Gross morphological examination of pituitary gland

In the AGR, the pituitary stalk or infundibulum attaches dorsally to the median eminence (*tuber cinereum*) and ventrally to the pituitary gland (Fig. 2). The pituitary gland of the AGR was also observed to be located in the hypophyseal fossa called *sella turcica* (Fig. 3A and 3B), which appeared as a very shallow depression on the sphenoid bone of the skull (Fig. 3C and 3D).

The pituitary gland in the AGR was somehow laterally extended with saddle-shaped and dorso-ventrally flattened (Fig. 4), and was enclosed with meninges which were serving as its capsule. The meninges were attached to it except at the upper part, where the pituitary stalk comes out and connects to the brain through median eminence of the hypothalamus (Fig. 2). The anterior pituitary (adenohypophysis) and posterior pituitary (neurohypophysis) lobes were distinguishable on physical examination (Fig. 4).

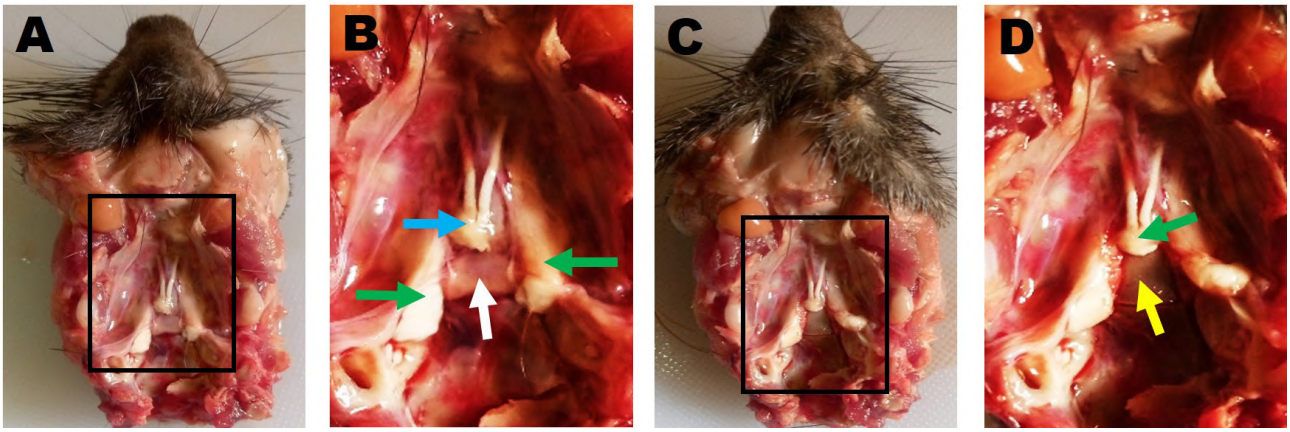

Fig. 3.- Photograph of head of AGR showing pituitary gland and other related structures after careful removal of the brain. **A, B:** The pituitary gland (white arrow) lying in the *sella turcica* on the basisphenoid bone. The optic nerve with small remnant of optic chiasm (blue arrow) is seen located rostral to the pituitary gland while the trigeminal nerve roots (green arrows) located at the lateral boundaries. **C, D:** The basisphenoid bone showing the *sella turcica* (yellow arrow) after the pituitary gland was removed.

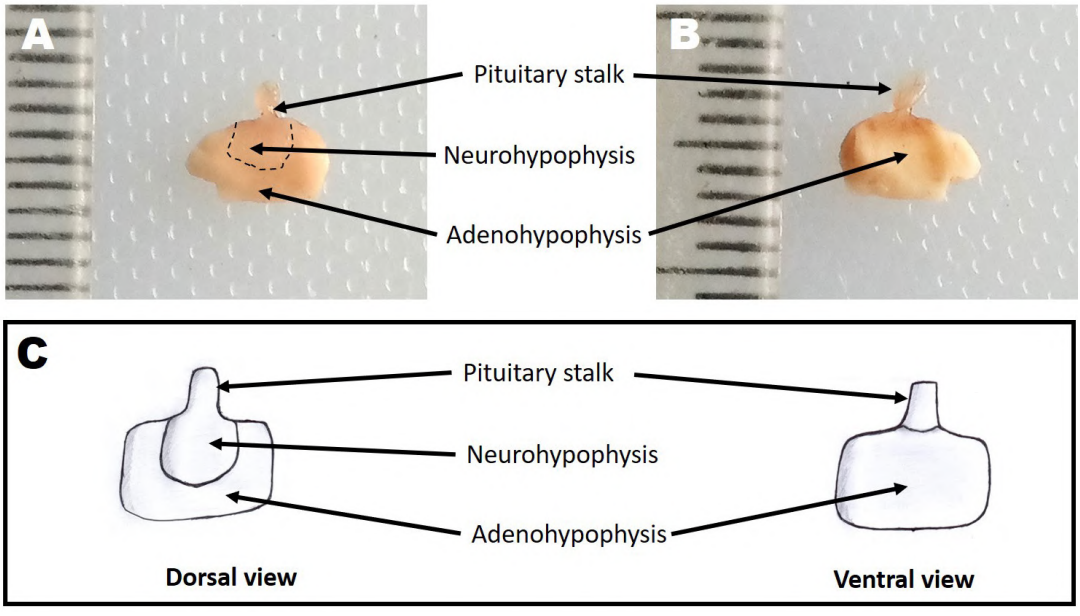

Fig. 4.- The photographs of AGR pituitary glands showing the infundibular stalk, adenohypophysis and neurohypophysis. **A:** Dorsal view of AGR pituitary gland. **B:** Ventral view of AGR pituitary gland. **C:** Schematic showing both the dorsal and ventral view of the AGR pituitary gland.

Grossly, the adenohypophysis was observed to be gray in colour and was greater in size than the neurohypophysis (Fig. 5), while the neurohypophysis was seen to be whitish in colour. There were no obvious differences on gross examination between the adult and juvenile groups.

The body, brain and pituitary gland weights

The mean weights of the body, brain and pituitary gland of the adult group in AGRs were 984.8 ± 34.09 g, 5.6 ± 0.18 g and 0.019 ± 0.0021 g respectively. In the juvenile, the mean weights of the body, brain and pituitary glands were 493.7 ± 23.41 g, 4.42 ± 0.11 g and 0.012 ± 0.0054 g respectively. The body weights were significantly ($P > 0.05$) higher in adult AGR (984.8 ± 34.09 g) when compared to juvenile (493.7 ± 23.41 g). The weights of brain were significantly ($P > 0.05$) higher in adult AGR (5.6 ± 0.18 g) when compared to juvenile (4.42 ± 0.11 g). The weights of pituitary glands were also significantly ($P > 0.05$) higher in adult AGR (0.019 ± 0.0021 g) when compared to juvenile (0.012 ± 0.0054 g).

The mean relative weight of the pituitary gland in adult AGR (0.0019 ± 0.0016 g) and young AGR (0.0023 ± 0.0022 g) is indistinguishable.

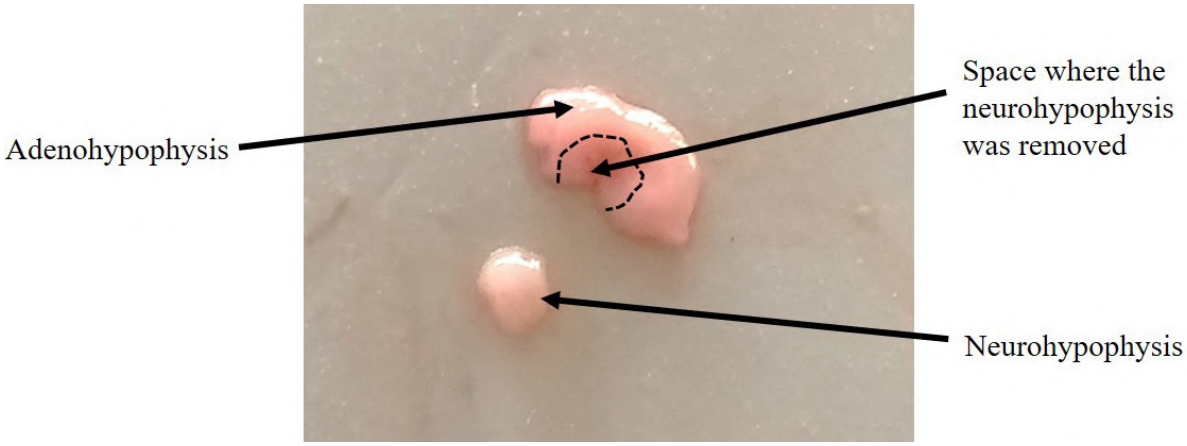

Fig. 5.- A photograph showing the adenohypophysis and neurohypophysis of the pituitary gland on physical examination.

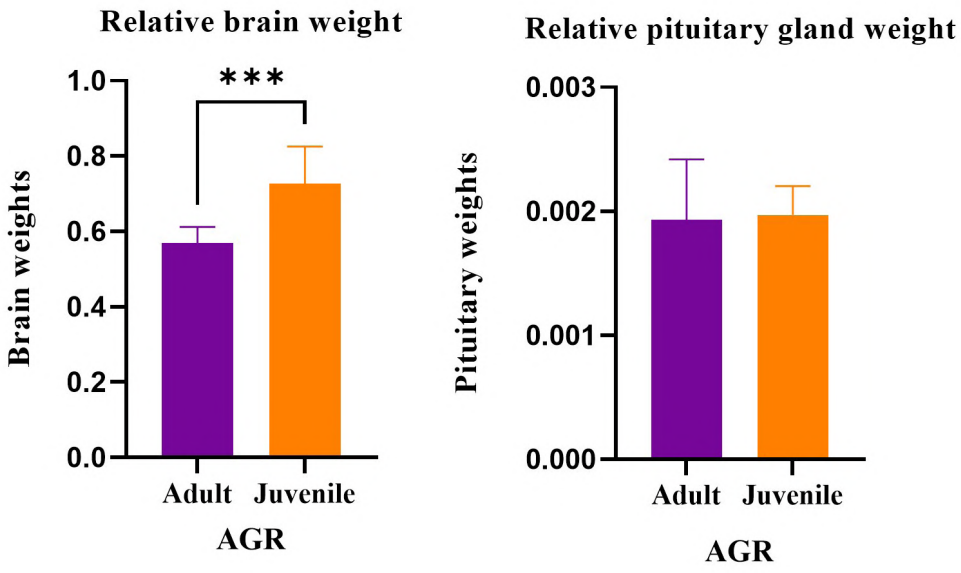

Fig. 6.- Bar diagrams showing the relative brain weight (A) and relative pituitary weight (B) of juvenile and adult African giant rat (AGR). Plots are mean ± SEM of relative organ weight. Student's t-test, ***$P < 0.001$. Statistical significance is indicated for adult and juvenile AGR brain weight data. No statistical difference for relative pituitary weight ($P<0.05$). Normality test (Shapiro-Wilk test) = relative brain weight (adult: P<value 0.903; juvenile P<value 0.171), relative pituitary weight (adult: P<value 0.317; juvenile P<value 0.899).

Table 2. Measurements of the length and width of the pituitary gland in AGR.

Parameters Group	Number of animals	Mean Pituitary Length (mm) Mean ± SEM	Mean Pituitary Width (mm) Mean ± SEM
Adult	9	4.105 ± 0.005	5.910 ± 0.006
Juvenile	6	3.515 ± 0.005	4.815 ± 0.005

SEM = standard error of mean; mm = millimeters; Normality test (Shapiro-Wilk test) = adult (length: P<value 0.954; width P<value 0.496), juvenile (length: P<value 0.0.6708; width P<value 0.067).

With increasing body weight, there was contemporaneous increase in the weight of the pituitary gland. The mean relative weight of the brain in the adult AGR (0.57 ± 0.014 g) was significantly lower than the mean relative brain weight in young AGR (0.73 ± 0.040 g). This implies that, as the body size is increasing, there is exponential increase in brain weight of young AGR. On the other hand, it appears that in the adult AGR the brain weight did not increase at the same rate as the body size was increasing. This was observed when the mean relative brain weight of adult AGR was compared to that of young AGR (Fig. 6). The normal distribution of the data was confirmed by employing normality test (Shapiro-Wilk test).

The length and width of the pituitary gland

The pituitary glands were generally wider (distance from their lateral edges) than they were longer (rostro-caudal distance). Grossly, the mean length of pituitary glands showed statistically significant (P > 0.05) variation between both the adult (4.105 ± 0.005 mm) and juvenile (3.515 ± 0.005 mm) groups. The length is higher in the adult when compared to juvenile. The width of the pituitary gland was also significantly (P < 0.05) higher in the adult (5.910 ± 0.006 mm) when compared to the juvenile group (4.815 ± 0.005 mm) (Table 2).

Histological examinations of the pituitary gland

The pituitary gland of the AGR consists of anterior lobe or adenohypophysis (*pars tuberalis, pars distalis*) and posterior lobe or neurohypophysis (*pars nervosa*), which are separated by the intermediate lobe (*pars intermedia adenohypophysis*) and hypophyseal cleft (Fig. 7A). The *pars tuberalis adenohypophysis* in AGR was identified together with pituitary

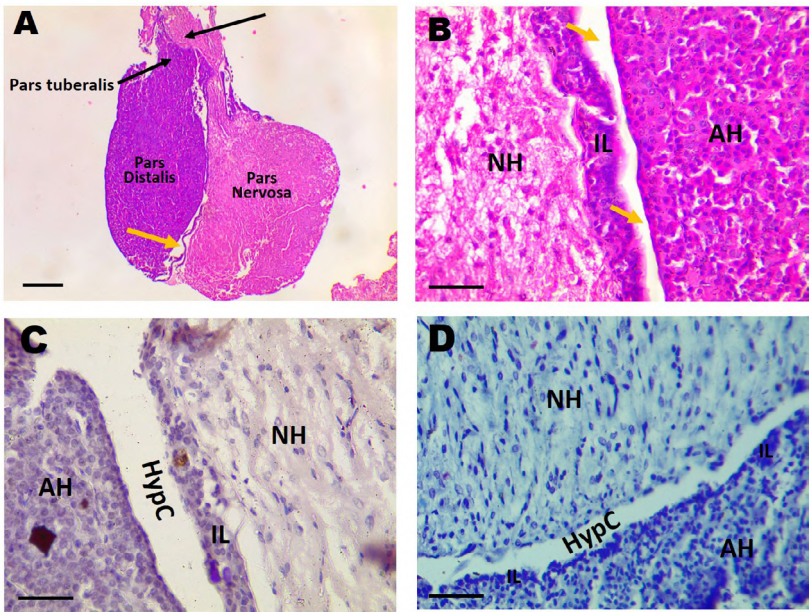

Fig. 7.- The pituitary gland of the AGR showing the adenohypophysis (pars distalis and pars tuberalis), neurohypophysis (pars nervosa), intermediate lobe (IL) showing melanotrophs (blue arrows), pituitary stalk and hypophyseal cleft (yellow arrow). NH: Neurohypophysis, AH: Adenohypophysis. HypC: Hypophyseal cleft. (**A:** H&E 4x, scale bar = 200 µm. **B:** H&E 40x, scale bar = 50 µm. **C:** PAS 40x, scale bar 50 µm. **D:** Cresyl violet 40x, scale bar 50 µm).

stalk (*infundibulum neurohypophysis*) (Fig. 7A), due to the difficulty in lifting the pituitary gland out of its hypophyseal fossa without rupturing the pituitary stalk. The melanotrophs or melanotrophic cells of intermediate lobe of AGR (Fig. 8) are well developed and were easily distinguished.

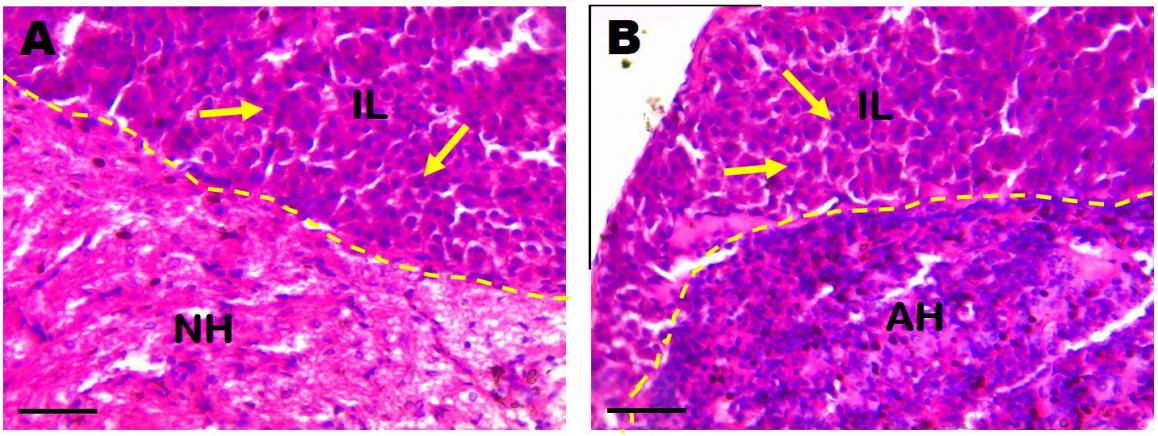

Fig. 8.- Micrographs of the pituitary gland (**A, B**) in the AGR showing the melanotrophs (yellow arrows) in the intermediate lobe (IL). NH: neurohypophysis, AH: adenohypophysis. (H&E 40x, scale bars = 50 µm).

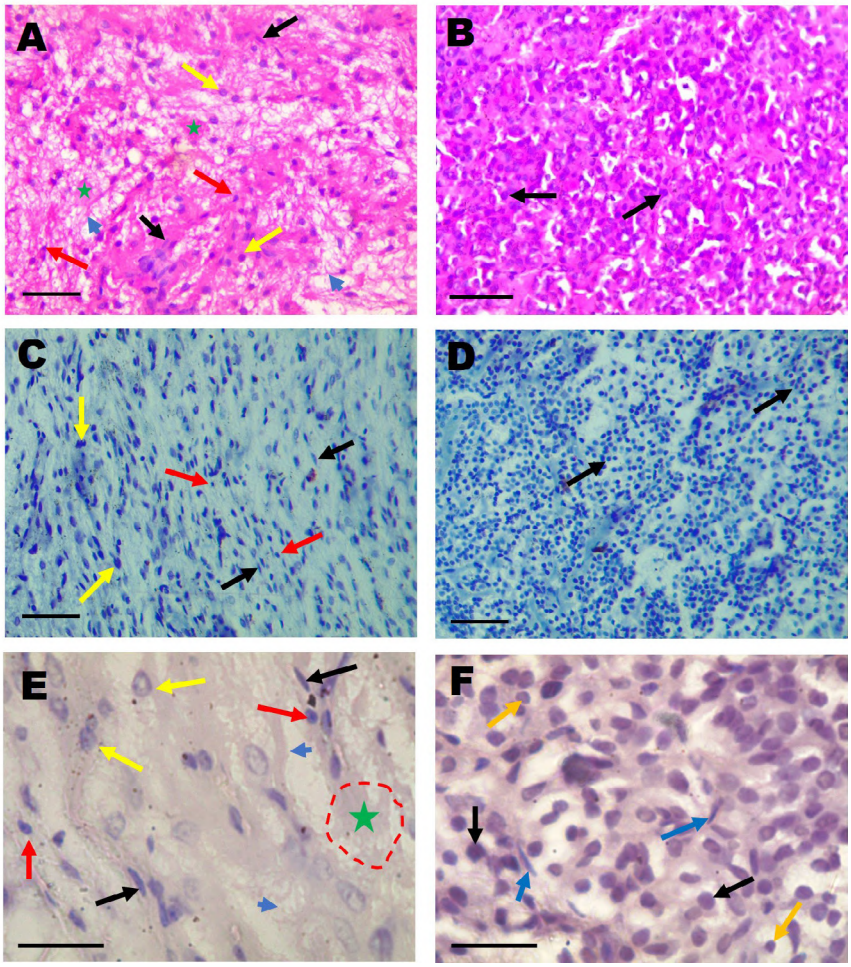

Fig. 9.- The neurohypophysis and adenohypophysis of AGR pituitary gland. **A, C, E:** The neurohypophysis of AGR pituitary gland showing the protoplasmic pituicyte (black arrow), fibrous pituicyte (yellow arrow), Herring bodies (green stars) and unmyelinated nerve fibres (blue arrowheads). In the neurohypophysis, three different pituicyte nuclei were identified: elongated shaped nuclei (black arrow), oval shaped nucleus (yellow arrow) and triangular shaped nucleus (green arrow). **B, D, F:** The adenohypophysis showing the chromophils (blue arrow) with lightly stained cytoplasm by eosin and chromophobes (black arrow) which are the biggest cells in adenohypophysis. (**A, B:** H&E 10x, scale bars = 25 µm. **C, D:** Cresyl violet 100x, scale bars = 50 µm. **E, F:** PAS 100x, scale bars = 25 µm).

Table 3. The mean hypophyseal length and width.

Parameters	Mean Hypophyseal Length (μm)		Mean Hypophyseal Width (μm)	
Group	Mean ± SEM		Mean ± SEM	
	Mean NL	Mean AL	Mean NW	Mean AL
Adult (n = 4	1218.0 ± 163.00	3663.0 ± 211.3	795.0 ± 105.00	1973.0 ± 0.00
Juvenile (n = 4)	403.0 ± 41.60	1621.0 ± 89.20	160.0 ± 17.70	885.0 ± 157.00

SEM = standard error of mean; n = number of experimental AGR used; μm = micrometers; NL = neurohypophyseal length; AL = adenohypophyseal length; NW neurohypophyseal width; AW adenohypophyseal width; Normality test (Shapiro-Wilk test) = mean NL (adult: P<value 0.262; juvenile P<value 0.995), mean NW (adult: P<value 0.957; juvenile P<value 0.850), mean AL (adult: P<value 0.726; juvenile P<value 0.849), mean AL (adult: P<value 0.574; juvenile P<value 0.661).

Chromophils and chromophobes, as the epithelial cells of the adenohypophysis, were identified in the adenohypophysis based on staining properties (Fig. 9). Here the neurohypophysis in AGR is made of nerve fibres and pituicytes, which are supportive astrocytic-like glial cells (Fig. 9). We were able to identify the two types of pituicytes: protoplasmic pituicytes, with elongated nuclei, and fibrous pituicytes, with round nuclei. Furthermore, based on nuclei shape, three kinds of pituicytes were also observed in the neurohypophysis. These are: oval-shaped nucleus, triangle-shaped nucleus and elongated nucleus (Fig. 9). The Herring bodies which are the neurosecretory bodies and non-myelinated nerve fibres extended from the hypothalamus were distinctly visible among the pituicytes in the neurohypophysis (Fig. 9).

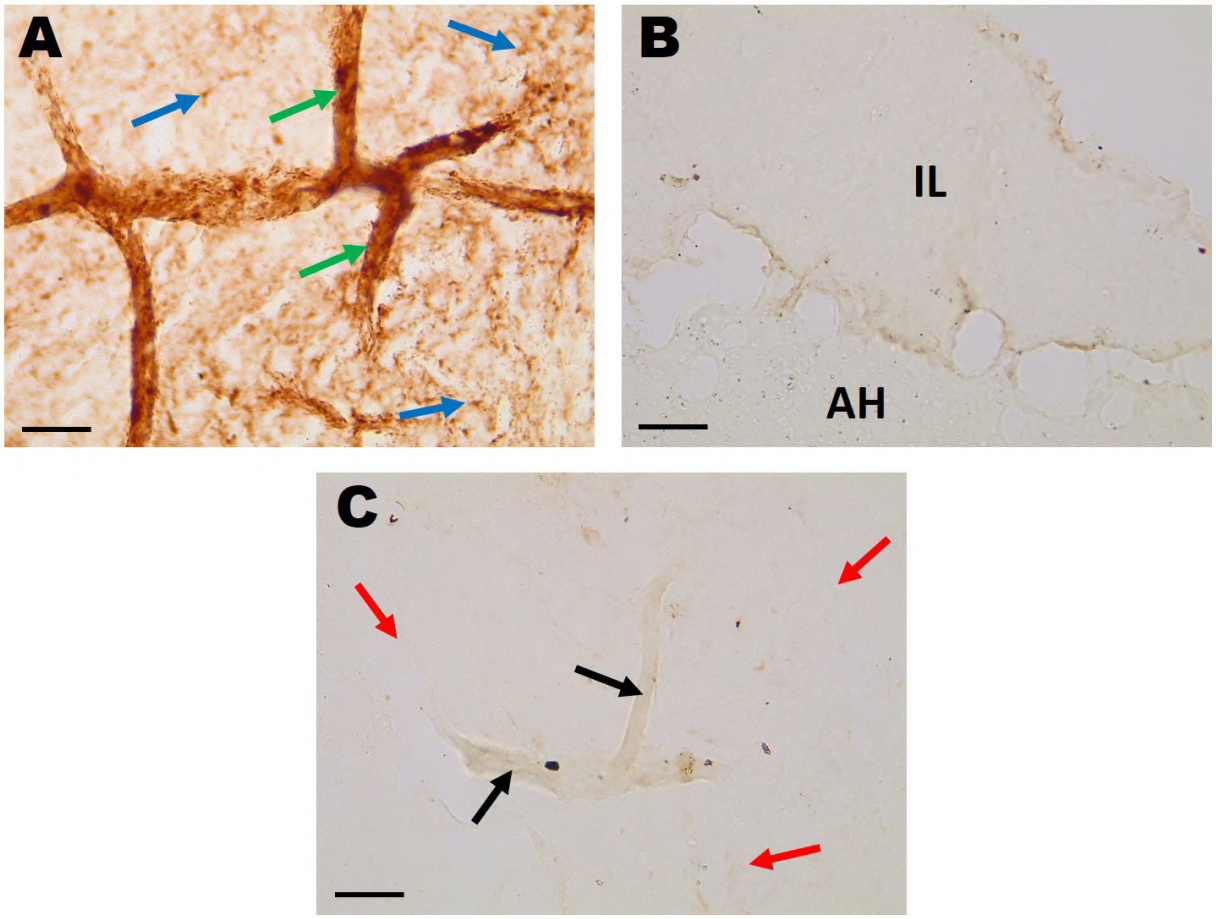

Fig. 10.- Micrographs of the pituitary gland showing GFAP immunohistochemistry in the AGR. **A:** The pituitary gland showing strong immunostaining of pituicytes (blue arrow) in the neurohypophysis. The blood vessels (green arrows) show stronger immunoreactivity to GFAP by the end-feet of the pituicytes. **B:** The gland showing the negative immunoreactivity by parenchyma of both intermediate lobe (IL) and adenohypophysis (AH). **C:** The adenohypophysis showing no immunolabelling to GFAP (red arrow). The black arrow is showing the blood vessels within the its parenchyma. (GFAP 40x, scale bars = 50 μm).

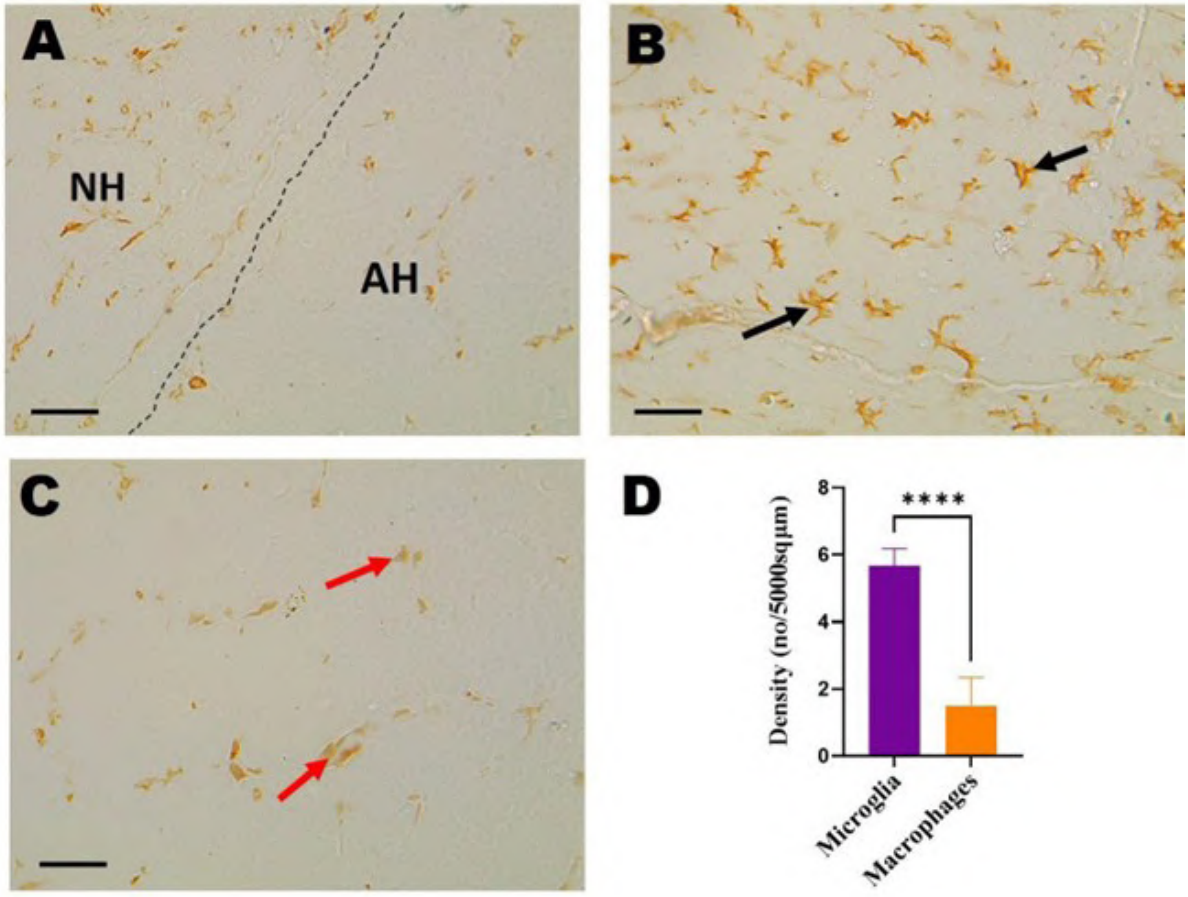

Fig. 11.- Micrographs showing immunohistochemistry of the pituitary gland of AGR to Iba1. **A:** The pituitary gland showing high immunoreactivity of microglia-like cells in the neurohypophysis as compared to adenohypophysis. **B:** The neurohypophysis showing a strong positive immunoreactivity of microglia (black arrow). **C:** Adenohypophysis revealing few macrophages (red arrow) that are immunostained positively to Iba1. **D:** Density of microglia and macrophages in the neurohypophysis and adenohypophysis respectively. Plots are mean ± SEM of microglia/macrophages density. Student's t-test, ****P<0.0001. Statistical significance is indicated for microglia/macrophages density. (Iba1 40x, Scale bars = 50 μm).

Histometric analysis of the pituitary gland

Mean adenohypophyseal length (Mean AL) in juvenile (1621±89.20 μm) and adult (3663.0 ± 211.3 μm) male AGR was noticeably longer than that of neurohypophysis (Mean NL) in juvenile (403.0 ± 41.60) and adult (1218±163.00 μm). Similarly, the mean adenohypophyseal width (Mean AW) in both juvenile (885±157.00 μm) and adult (1973±00.00 μm) was wider than the neurohypophyseal width (Mean NW) in juvenile (160.00 ± 17.70 μm) and adult (795.00 ±105.00 μm) (Table 3).

Immunohistochemical assessment

In AGR pituitary glands, the pituicytes showed strong immunostaining with GFAP antibody in the neurohypophysis, whereas in the adenohypophysis and intermediate lobe there was no immunochemical expression of the same (Fig. 10).

The pituitary gland expressed a stronger positive immunoreactivity to microglia in the neurohypophysis as compared to macrophages in the adenohypophysis. The adenohypophysis revealed the expression of few macrophages that were positively immunolabeled with Iba1 antibody (Fig. 11). The density ratio of microglia present in the neurohypophysis of the AGR pituitary gland was significantly higher than macrophages in the adenohypophysis.

DISCUSSION

Our findings showed that pituitary glands in AGR varies with age which agrees with the reports of Maskey et al. (2021), they reported that the pituitary gland size differs with age and gender. Mixer and Turner (1942) have also reported increased in pituitary weight with corresponding increase

in body weight among male growing albino rats. The late age increase in pituitary length and width was mainly attributed to the increment of gonadotropic hormones due to loss of feedback mechanism of gonadal steroids. However, Luay (2016) reported figures in the guinea pig that appear to be too high when compared to AGR, which is also a large rodent like guinea pig.

Grossly, the pituitary gland of the AGR was located at the base of the brain, on the sphenoid bone, as described for domestic mammals (Dyce et al., 2010). The gland was in an ill-defined depression of the sphenoid bone called *sella turcica*. Our results corroborated those of Mahmood (2014), who described the anatomical and histological features of the pituitary gland in rats and found that, for most investigated species, there was no true *sella turcica*, but rather, a shallow depression in the sphenoid bone. The gland is saddled in shape, contrary to the reports of Mahmood (2014) in rats and Gilbert et al. (2020) in greater cane rats, who both reported a disc shape. The pituitary gland was also seen between the two roots of the trigeminal nerve of AGR as reported by Cao et al. (2017) in mice and Gilbert et al. (2020) in greater cane rats.

Histologically, the neurohypophysis is also considerably smaller than the adenohypophysis, which confirmed previously published data by Mahmood (2014) in rats and Gilbert et al. (2020) in greater cane rats. In this study we also found that the adenohypophysis and the neurohypophysis were in contact and joined through the intermediate lobe, as reported by Mahmood (2014) in rats. This intermediate lobe is absent in the avian species, as reported in turkey by Jahangirfard et al. (2019). Dyce et al. (2010) reported that the intermediate lobe is poorly developed in the primates. Furthermore, Perez-Castro et al. (2012) described that the pituitary gland of humans lacks the intermediate lobe and the hypophyseal cleft.

Our study revealed two types of parenchymal cells in the adenohypophysis. These are: chromophils and chromophobes. This agrees with the reports of Gilbert et al. (2020), where they identified chromophilic acidophils, chromophilic basophils and chromophobes in the pituitary glands of the greater cane rats. These cells in adenohypophysis were seen to be arranged in cords, as reported by Mahmood (2014). Using H&E, the neurohypophysis revealed two types of pituicytes: fibrous and protoplasmic pituicytes, as described by Ye et al. (2018) in camels. Furthermore, three types of pituicyte nuclei were identified in the AGR neurohypophysis: oval-shaped nucleus, triangle-shaped nucleus and elongated nucleus. These types of pituicyte nuclei shapes were also described by Mahmood (2014) and Gilbert et al. (2020).

The strong expression of pituicytes with GFAP antibody in the AGR neurohypophysis was possible because pituicytes are the astrocyte-like cells present in the neurohypophysis. Gilbert et al. (2020) also demonstrated pituicytes immuno-labelled by GFAP antibody in the greater cane rats (GCR). There was positive immunoreactivity of microglia and macrophages to Iba1 antibody in the neurohypophysis and adenohypophysis respectively. This has been corroborated by reports of Mander and Morris (1996), where they demonstrated the presence of neurohypophyseal microglia and adenohypophyseal macrophages in rats. This is contrary to the reports of Gilbert et al. (2020), in which a negative immunostaining to Iba1 antibody both in the neurohypophysis and adenohypophysis of the greater cane rat (GCR) was reported (Gilbert et al., 2020). This may be attributed to species specificity. The density of microglia in the AGR was seen to be higher than macrophages in the adenohypophysis. Mander and Morris (1996) reported that microglia in the neurohypophysis constituted 20% of the non-endothelial cells with the other 80% of cells being pituicytes. Mander and Morris (1996) reported macrophages to be only 1% of the total cells in the adenohypophysis. This current study only considered male AGR, which invariably introduce gender bias – a limitation of this research. We plan to investigate the architecture of the pituitary gland in female AGR for comparative study with regard to sexual dimorphism.

In conclusion, the weight, length and width of the pituitary glands in AGRs increases with age. The gross and histological characteristics of pituitary glands of the AGR are found to be similar to other rodents and mammals in general. We recommend further study to compare morphometrical parameters between males and females of this model.

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REFERENCES

ACOSTA M, MOHAMED F (2013) Immunohistochemical and morphometric study of pituitary pars distalis folliculostellate cells of nonpregnant and pregnant viscachas. *Biotechnic Histochem*, 88(3-4): 161-169.

AJAYI SS (1974) The biology and domestication of African giant rat (*Cricetomys gambianus*, Waterhouse), Ph.D. Thesis. University of Ibadan, Nigeria.

ANGELA N, VIAENE AN, LEE EB, ROSENBAUM JN, NASRALLAH IM, NASRALLAH MP (2019) Histologic, immunohistochemical, and molecular features of pituicytomas and atypical pituicytomas. *Acta Neuropath Comms*, 7: 6.

ACOSTA M, MOHAMED F (2009) Pituitary pars intermedia of male viscacha (*Lagostomus maximus maximus*): a morphometric study of seasonal and age-related changes in immunohistochemistry. *Cells Tissues Organs*, 190: 219-229.

AMAR AP, WEISS MH (2003) Pituitary anatomy and physiology. *Neurosurg Clin N Am*, 14: 11-23.

AMAT P (1970) Thyrotroph subtypes in the guinea-pig's pituitary after partial and total thyroidectomy. *Cells Tissues Organs*, 76(1): 112-122.

BOTERMANN DS, BRANDES N, FROMMHOLD A, HEß I, WOLFF A, ZIBAT A, HAHN H, BUSLEI R, UHMANN A (2021) Hedgehog signaling in endocrine and folliculo-stellate cells of the adult pituitary. *J Endocr*, 248: 303-316.

CARDIN J, CARBAJAL ME, VITALE ML (2000) Biochemical and morphological diversity among folliculo-stellate cells of the mink (*Mustela vison*) anterior pituitary. *Gen Comp Endocrinol*, 120: 75-87.

CÓNSOLE GM, JURADO SB, OYHENART E, FERESE C, PUCCIARELLI H, GÓMEZ DUMM CLA (2001) Morphometric and ultrastructural analysis of different pituitary cell populations in undernourished monkeys. *Braz J Med Biol Res*, 34(1).

CAO D, MA X, ZHANG WJ, XIE Z (2017) Dissection and coronal slice preparation of developing mouse pituitary gland. *JOVE*, 129: e56356.

DYCE KM, SACK WO, WENSING CJG (2010) Textbook of veterinary anatomy, 4th edition. Saunders. An Imprint of Elsevier Inc, St Louis, Missouri, pp 268-290.

EMERALD M (2016) Pituitary Gland: Pituitary Hormones. *Encycl Food Heal*, pp 392-400.

FREEMAN AR, OPHIR AG, SHEEHAN MJ (2020) The giant pouched rat (*Cricetomys ansorgei*) olfactory receptor repertoire. *PloS One*, 15(4): e0221981.

GILBERT TT, OLOPADE FE, MUSTAPHA OA, FOLARIN OR, OLOPADE JO (2020) Histological and immunohistochemical study of pineal and pituitary glands of the greater cane rat (*Thryonomys swinderianus*, Temminck 1827). *Arch Bas App Med*, 8: 137-142.

JAHANGIRFARD R, SHALIZAR-JALALI A, SHAHROOZ R, NAJAFI G, MINAS N (2019) Anatomical and cytohistological study of the pituitary gland in adult turkey. *Vet Res For*, 10(2): 159-163.

JU K, BAE H, PARK H, CHANG J, CHOI KS, SIM K (2010) Morphometric study of the Korean adult pituitary glands and the diaphragma sellae. *J Korean Neurosurg Soc*, 47: 42-47.

KAUFMAN M, NIKITIN AY, SUNDBERG JP (eds.) (2009) *Histologic basis of mouse endocrine system development: a comparative analysis.* CRC Press, Boca Raton.

KONDOH D, NAKAMURA KG, ONO YS, YUHARA K, BANDO G, WATANABE K, HORIUCHI N, KOBAYASHI Y, SASAKI M, KITAMURA N (2017) Histological features of the vomeronasal organ in the giraffe, *Giraffa cameloparadalis. Microsc Res Tech*, 80(6): 652-656.

LAMICHHANE TR, PANGENI S, PAUDEL S, LAMICHHANE HP (2015) Age and gender related variations of pituitary gland size of healthy Nepalese people using magnetic resonance imaging. *Am J Biomed Engineer*, 5(4): 130-135.

LUAY OH (2016) Some macroscopic and microscopic observation on the pituitary gland of guinea-pig *Cavia culteri. Bas J Vet Res*, 15(4).

MANDER TH, MORRIS JF (1996) Development of microglia and macrophages in the postnatal rat pituitary. *Cell Tissue Res*, 286: 347-355.

MCKLVEEN TL, JONES JC, SPONENBERG DP, KENT S, WARD DL, AARDEMA CH (2003) Assessment of the accuracy of computed tomography for measurement of normal equine pituitary glands. *Am J Vet Res*, 64(11): 1387-1394.

MASKEY S, MANSUR DI, KARKI S, SHRESTHA P, SHRESTHA S, MUKTA SINGH BHANDARI MS (2021) Morphometric study of pituitary gland with correlation of age and gender using Magnetic Resonance Imaging. *BJHS*, 6(2): 1476-1480.

MAHMOOD HB (2014) Anatomical and histological study of pituitary gland of the rats in Iraq. *J Kerb Uni*, 12: 221-228.

MUSTAPHA OA, OLUDE MA, TAIWO B, OLOPADE JO (2019) Cytoarchitecture of the hippocampal formation in the African giant rat (*Cricetomys gambianus*, Waterhouse). *Nigerian J Physiol Sci*, 34(1): 55-62.

MONTEMURRO DG (1964) Weight and histology of the pituitary gland in castrated male rats with hypothalamic lesions. *J Endocr*, 30(1): 57-67.

MIXNER JP, TURNER CW (1942) Pituitary weight of growing male albino rat related to body weight. *Endocrinology*, 31(2): 261-263.

NZALAK JO, AYO JO, NEILS JS, OKPARA JO, ONYEANUSI BI, GHAJI A, OJO SA, ADEBAYO IA (2005) Morphometric studies of the cerebellum and forebrain of the African giant rat (AGR) (*Cricetomys Gambianus*, Waterhouse). *Trop Vet*, 23(3-4): 87-92.

OLOPADE F, FEMI-AKINLOSOTU OM, ADEKANMBI AJ, IGHOGBOJA OO, SHOKUNBI MT (2021) Chronic caffeine ingestion improves memory and learning and increases neuronal population and dendritic length in the hippocampus of adult mice. *Nigerian J Physiol Sci*, 36(2): 165-172.

OLOPADE JO, FATOLA IO, OLOPADE FE (2011) Vertical administration of vanadium through lactation induces behavioural and neuromorphological changes: protective role of vitamin E. *Nigerian J Physiol Sci*, 26(1): 55-60.

OLUDE MA, OGUNBUNMI TK, OLOPADE JO, IHUNWO AO (2014) The olfactory bulb structure of African giant rat (*Cricetomys gambianus*, Waterhouse 1840) I: cytoarchitecture. *Anat Sci Int*, 89(4): 224-231.

OLUDE MA, MUSTAPHA OA, OLOPADE JO (2016) Morphological characterization of the African giant rat (*Cricetomys gambianus*, Waterhouse) brain across age groups: gross features of cortices. *Nigerian J Physiol Sci*, 31: 133-138.

PEREZ-CASTRO C, RENNER U, HAEDO MR, STALLA GK, ARZT E (2012) Cellular and molecular specificity of pituitary gland physiology. *Physiol Rev*, 92: 1-38.

POPOOLA SO, SAKPA CL (2018) Hormones of pituitary-gonadal axis and histology of pituitary gland following oral treatment of male Wistar rats with glyphosate. *J Biomed Res Clin Pract*, 1(2).

RADHIAH NA, AZHAR LJ (2020) Effect of evisect on organo-somatic index and pathohistological changes of some vital organs in white mice. *Sys Rev Pharm*, 11(11): 1910-1914.

RAHMAN M, ARA S, AFROZ H, HAHAR N, SULTANA AA, FATEMA K (2011) Morphometric study of the human pituitary gland. *Bangladesh J Anat*, 9(2): 79-83.

SCHREIBMAN MP, LEATHERLAND JF, MCKEOWN BA (1973) Functional morphology of the teleost pituitary gland. *Amer Zool*, 13: 719-722.

SCHOEMAKER NJ, VAN DER HAGE MH, FLIK G, LUMEIJ JT, RIJNBERK A (2004) Morphology of the pituitary gland in ferrets (*Mustela putorius furo*) with hyperadrenocorticism. *J Comp Path*, 130: 255-265.

TREIER M, O'CONNELL S, GLEIBERMAN A, PRICE J, SZETO DP, BURGESS R, CHUANG PT, MCMAHON AP, ROSENFELD MG (2001) Hedgehog signaling is required for pituitary gland development. *Development*, 128: 377-386.

VIONNA K, KATHERINE Q, ROUX L, ALEXANDRE B (2020) Mensuration of the rabbit pituitary gland from computed tomography. *Vet Radiol Ultrasound*, 61(3): 322-328.

WEMAN B (1974) Fine structure of the pars distalis of the pituitary gland in the female mink, *Mustela vison*. *Acta Zoologica*, 55(2): 119-136.

WEMAN B, NOBIN A (1973) The pars intermedia of the mink, *Mustela vison*. *Z Zellforsch*, 143(3): 313-327.

WITTKOWSKI W (1998) Tanycytes and pituicytes: morphological and functional aspects of neuroglial interaction. *Microsc Res Tech*, 41: 29-42.

YE W, WANG H, WANG F, WANG J (2018) Morphology and ultrastructure of the hypophysis in Bactrian Camels (Camelus bactrianus). *Int J Morphol*, 36(4): 1316-1323.