



# Morphological Assessments and Sexual Dimorphism of the Visual Pathway Structures of *Cricetomys gambianus* (African Giant Rats Waterhouse-1840)

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## Abstract

**BACKGROUND AND AIM:** *Cricetomys gambianus* (African Giant Rat (AGR) a nocturnal rodent has recently been a choice model for several areas of neuroscience research. This study assessed sexual differences in the morphometric parameters of visual pathway structures of the AGR.

**MATERIALS AND METHODS:** Ten healthy adult AGRs (n=5; Male (M) and Female (F)) were captured from the wild and used for this study. The AGRs were sedated and body weight obtained before perfusion and decapitation. The weight and dimensions of the brain, eyeballs, optic nerve, optic chiasma and optic tract were obtained using digital weighing balance and Vernier caliper, respectively. Data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) version 23. Results were expressed as mean  $\pm$  SEM.

**RESULTS:** Average body weight of AGRs was  $787 \pm 208.6$  g for males and  $926 \pm 133.0$  g for females; Brain weight was  $6.7 \pm 0.6$  g for males and  $6.9 \pm 0.6$  g females; while eyeball weight was  $0.23 \pm 0.02$  g for males and  $0.22 \pm 0.02$  g for females. Significant ( $p < 0.05$ ) sexual differences were observed in the dimensions of eyeball antero-posterior diameter (M= $7.19 \pm 0.24$  mm; F=  $6.56 \pm 0.17$  mm); Eyeball right-left diameter (M= $6.12 \pm 0.11$  mm; F=  $5.27 \pm 0.19$  mm); Optic nerve length (M= $2.18 \pm 0.18$  mm; F= $1.42 \pm 0.13$  mm); Optic tract length (M= $1.21 \pm 0.16$  mm; F= $1.02 \pm 0.08$  mm); Optic chiasma antero-posterior length (M= $1.02 \pm 0.24$  mm; F= $0.66 \pm 0.05$  mm) and Optic chiasma right-left length (M= $0.80 \pm 0.07$  mm; F= $0.73 \pm 0.05$  mm).

**CONCLUSION:** There exists sexual dimorphism in the morphometric parameters of visual pathway structures in the AGR. These differences are suggestive of evolutionary advantage in males compared to females as they go out in search for food and easy escape from predators within their natural habitat.

## Keywords:

Eyeball, Dimensions, Optic chiasma, Optic nerve, Optic tract

## INTRODUCTION

Sexual dimorphism is a common phenomenon in mammalian species (Lindenfors *et al.*, 2007; Shine, 2019). Evolutionarily, biological and environmental factors are critical in influencing specific patterns and extent of sexual dimorphism across species. Differences in bodily structures may contribute to sex-specific activity and associated with behavioral patterns in the species' natural habitat (Kappeler, 1991; Bimova *et al.*, 2018). Rodent species are important research tools in the biomedical field and have been reported to exhibit varying patterns of sex-based differences in the structures of the body including sensory organs (Campi and Krubitzer, 2010; Prendergast *et al.*, 2014).

Under-explored rodent species such as *Cricetomys gambianus* (African Giant Rat, AGR) has of recent been a choice model for several areas of neuroscience research, due to severally shared anatomical and physiological similarities with higher mammals including humans, particularly in their nervous system and sensory processing mechanisms (Jones, 2007; Okoye *et al.*, 2019). AGRs are nocturnal rodents which belongs to the family *Nesomyidae* and order *Rodentia*. They live in a variety of habitats ranging from arid to temperate areas (Ajayi, 1977; Igbokwe *et al.*, 2017). The AGRs are nearly inactive during the day, and come out at night in search of food. They are omnivorous, feeding on vegetables, insects, crabs, snails, and other items (Perry *et al.*, 2006). Both sexes are very territorial, but huddle together when temperature drop due to their low body fat as they do not retain heat easily

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(Nowak, 1997). The AGRs display a wide range of behavioral patterns in their natural habitat such as burrowing, foraging, and social interactions. These patterns may differ between sexes and could be useful in studying structures involved in neurological processes including sense of vision (Ajayi, 1977; Kingdon, 1997; Olude *et al.*, 2014).

The morphology of the visual pathway structures is conserved across evolutionary trend in mammalian species (Kaas, 2008; Campi and Krubitzer 2010); performing the function of receiving, relaying, and ultimately processing visual information. These structures include the eyeball, optic nerves, chiasm, tracts, lateral geniculate nucleus (LGN) of the thalamus, radiations and visual cortex (striate cortex, and extrastriate association cortices) (Felleman and Van Essen 1991). The eye is the primary sensory organ for vision, responsible for collecting light, focusing it, and encoding the first neural signals of the visual pathway. As soon as the visual information is generated in the retina as an appropriate physical stimulus, the electrical signals are conveyed to the brain via retinal ganglion cells (RGC) axons, which form the optic nerve as they exit the eye (Kaas, 1997; Sherman and Guillery, 2002). The optic nerve is an extension of the central nervous system (brain). The optic nerves from the two eyes decussates at the optic chiasma located at the base of the brain (Kaas, 2008).

Understanding the sex-based differences in the visual system of AGR could be beneficial in improving design and interpretation of visual and related neurological studies, crucial for the reliability and translational relevance of research using this species (AGR) as a model. A few studies have demonstrated sex differences in the morphometrics (size and structural measurements) of the visual pathway structures in some rodent species and associated to visual processing and function (Zilles *et al.*, 1984; Hua *et al.*, 2015). Hence, this study assessed sexual differences in the morphometric parameters of visual pathway structures of the AGR.

## MATERIALS AND METHODS

### Experimental Animals

Ten adult AGRs (n=5; male and female) were captured alive from the wild around Samaru Village in Zaria, Kaduna State, Nigeria. Using locally made metal cages, the AGRs were transported to the Neuroanatomy Laboratory, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, where they were acclimatized for 3 days before the commencement of the study (Conour *et al.*, 2006). During this period, the AGRs were allowed free access to drinking water and food (water melon, bean cake and ground nuts).

### Animal Euthanasia and Sample Collection

The AGRs were anesthetized with chloroform and the absolute body weights obtained using a digital electronic balance (Citizen Scales (1) PVT Ltd., U.S.A, sensitivity: 0.01 g). Thereafter, the AGRs were perfused transcardially; first, with normal saline to do a vascular rinsing, followed by 10% Buffered Formal Saline as described by Gage *et al.* (2012) and Ivang *et al.* (2023).

The whole brain, eyeballs and other structures of the visual pathway (optic nerves, chiasma and tracts) were carefully dissected by removal of cranial and facial bones. Enucleation of the eyeball was carried out according the method described by Mahajan *et al.* (2010). Enucleation involved the eyelids (superior and inferior) pulled apart to expose and access the eyeball. A curved dressing forceps was placed behind the globe in the orbit (eye socket). The forceps was closed to grasp the orbital connective tissue and optic nerve behind the globe while being careful to avoid squeezing the globe, and then pulled gently upward to extract the eyeball from the orbital cavity.

The harvested brain including structures of the visual pathway were observed for morphologic features and dimensions measured (see Figure 1).

### Gross Morphological Observations

The gross features of the AGR brain, eyeballs and other structures of the visual pathway were examined with the aid of a handheld lens (30 mm FHK Handheld Portable Magnifying Glass - China). Brain shape and colour, and other structures were observed on the dorsal and ventral brain surfaces. Definitions of gross anatomical structures were based on standard information on rodent anatomy (Rowet, 1979; Suckow *et al.*, 2006).

### Morphometric Assessments

The harvested whole brains of the AGR were weighed using a digital weighing scale (Mettler balance P 1210, Mettler instrument AG, Switzerland; sensitivity: 0.001) and, organosomatic (brain-body ratio) index were computed as described by Amber *et al.* (2020). Weight of the eyeballs (right and left) were measured and eyeball indices (eyeball-body weight and eyeball-brain weight ratios) were computed. Using a digital Vernier caliper (150 mm, China), the eyeball (right and left) dimensions measured were: Eyeball Antero-posterior (AP) - rostro-caudal diameter and Eyeball Right-left (R-L) diameter (see Figure 2).

The dimensions of the optic nerve, chiasma and tract (Optic Nerve Length; Optic Chiasma Right-Left Length; Optic Chiasma Antero-Posterior Length; Optic Tract Length) were obtained using non stretchable laboratory thread, a transparent 30 cm ruler and digital Vernier caliper.

## Data Analysis

Data collected on measured parameters were expressed as mean  $\pm$  SEM and subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 23.0. Student's *t*-test was used to determine the significance of the differences in the values obtained in AGR males and females. Values of  $p < 0.05$  were considered significant.

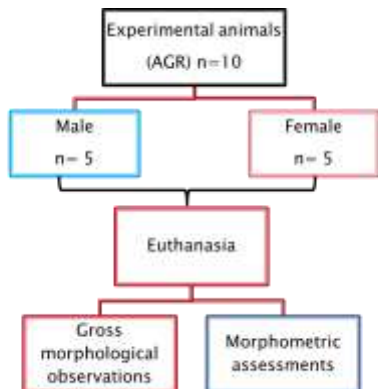


Figure 1: Experimental design

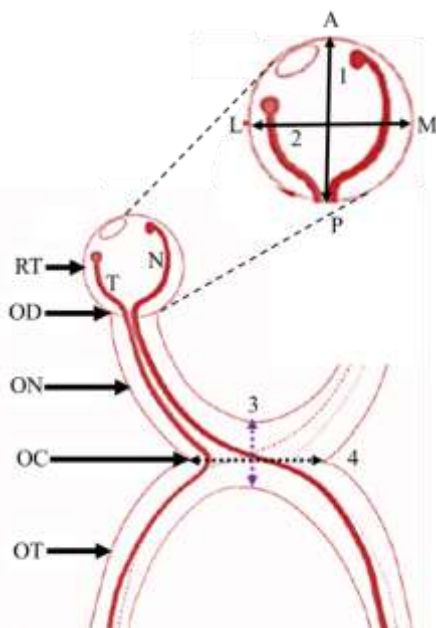


Figure 2: Measurement of dimensions of the visual pathway structures. Optic nerve (ON); Optic chiasma (OC); Optic tract (OT); Retina (RT); Optic disc (OD); Anterior (A); Posterior (P); Medial (M); Lateral (L); 1= Antero-posterior (AP) length of eyeball (EB); 2= Medio-lateral length of EB; 3= AP length of OC; 4= Right-to-left length of OC. **Indices:**  $EB/BrW \times 100$ ;  $BrW/BW \times 100$ ;  $EB/BW \times 100$  (Amber et al., 2020; Ivang et al., 2023) BrW = Brain Weight; BW = Body Weight; EB Eyeball.

## RESULTS

### Morphological Assessments

The absolute body weight, gross features of the brain, eyeballs and other structures of the visual pathway of AGRs were examined and are reported as follows: The average absolute body weight of AGRs showed higher values in females ( $926 \pm 133.0$  g) compared to their male ( $787 \pm 208.6$  g) counterparts. However, this difference was not significant (Figure 3A).

Gross observation of the AGR whole brain revealed a milky colour with an oval shaped orientation from a dorsal view. The brain dorsal surface presented with distinct parts including the cerebrum; the largest part of the brain, which lies immediately caudal to the olfactory bulb, rostral to the cerebellum and dorsal to the brain stem. On the ventral surface, the olfactory bulbs presented as rostral outgrowths of the brain, with the eyeballs (spherical and pigmented) laterally placed. Caudally, the paired optic nerves emerged from the eyeball, decussated at the optic chiasma and gives off optic tracts. Caudal to the optic tracts is the hind brain which comprised of the medulla oblongata, pons and cerebellum. The pons and medulla formed portions of the brain stem (Figure 4).

### Morphometric assessments

The mean values of AGR brain weight were less for the males ( $6.7 \pm 0.6$  g) compared to the females ( $6.9 \pm 0.6$  g) with no significant ( $p > 0.05$ ) difference (Figure 3B). Conversely, the organosomatic index revealed higher values ( $p > 0.05$ ) in males than their female counterparts (Figure 4).

The weight, dimensions and indices of the eyeballs of the male and female AGRs revealed asymmetry and sexual differences. The eyeball weight (right and left) showed higher values ( $p > 0.05$ ) in males than their female counterparts (Table 1). Relative to the dimensions (AP and R-L lengths) of the eyeball, sexual ( $p < 0.05$ ) differences were observed only in the right eyeball (Table 1).

The eyeball indices (eyeball-body weight and eyeball-brain weight ratios) showed significant sexual differences with males having higher values than their female counterparts (Figures 5B and 5C).

The male AGRs revealed higher values in the dimensions of the optic nerve, chiasma and tract compared to their female counterparts. These sexual differences were significant in all the parameters except, optic tract (Figure 6).

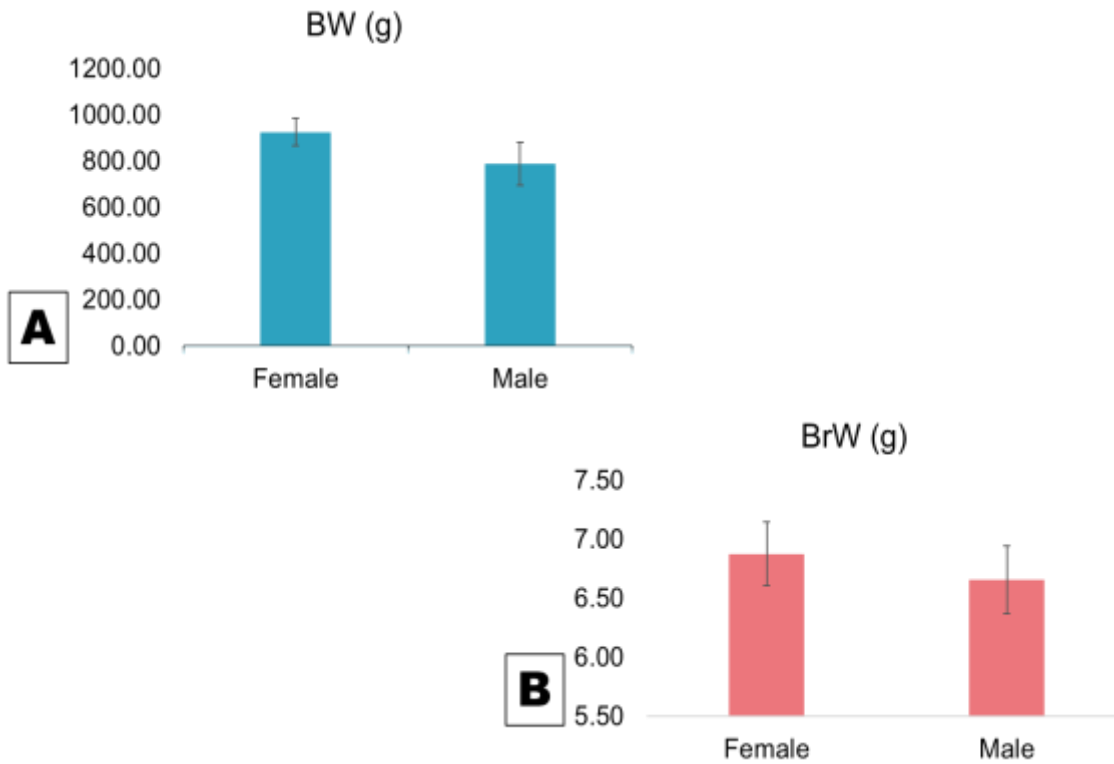


Figure 3: Comparison of body and brain weight of AGRs

BW = Body Weight; BrW = Brain Weight; n= 5; mean ± SEM; Unpaired t-test:  $p > 0.05$  (no significant difference)

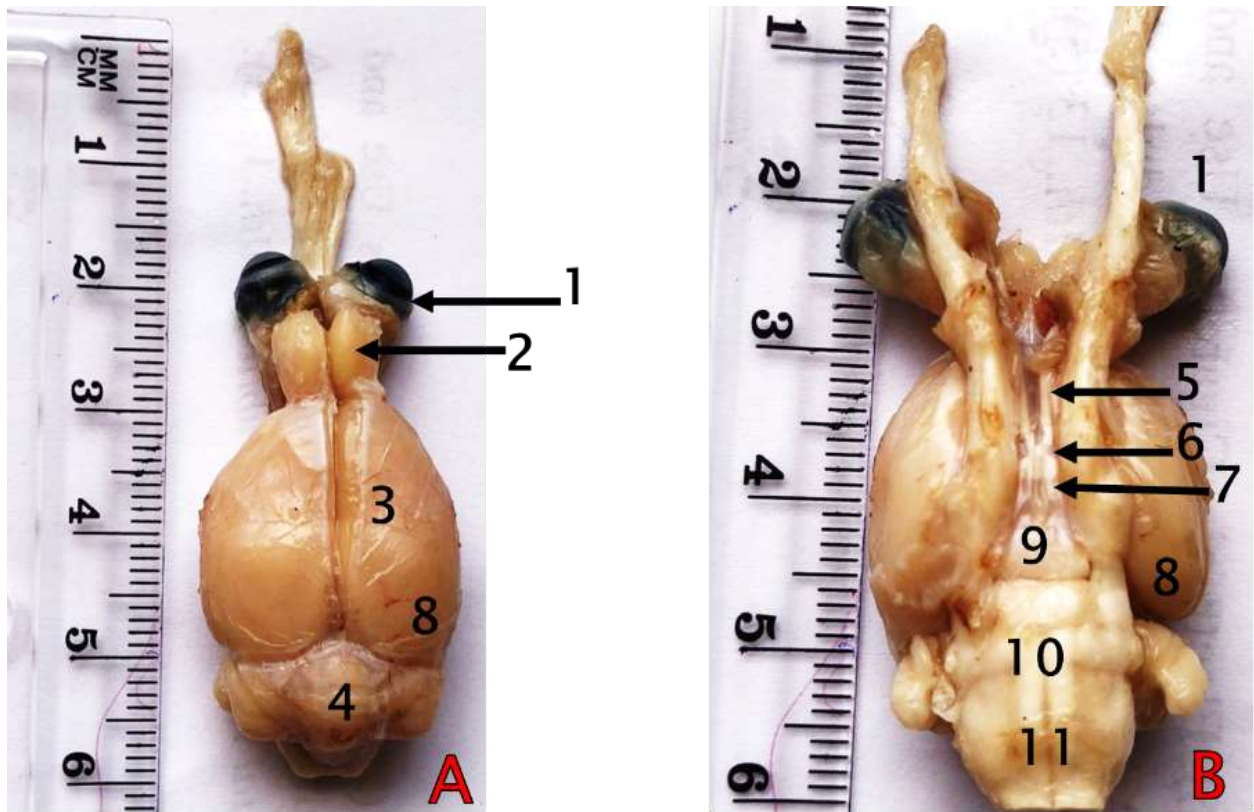


Figure 4: Gross morphological features of the AGR Brain; A= Dorsal, B = Ventral views.

1= Eyeball, 2= Olfactory bulb, 3= Cerebrum, 4= Cerebellum, 5= Optic Nerve, 6= Optic Chiasma, 7= Optic Tract, 8= Visual Cortex, 9= Midbrain, 10= Pons, 11= Medulla



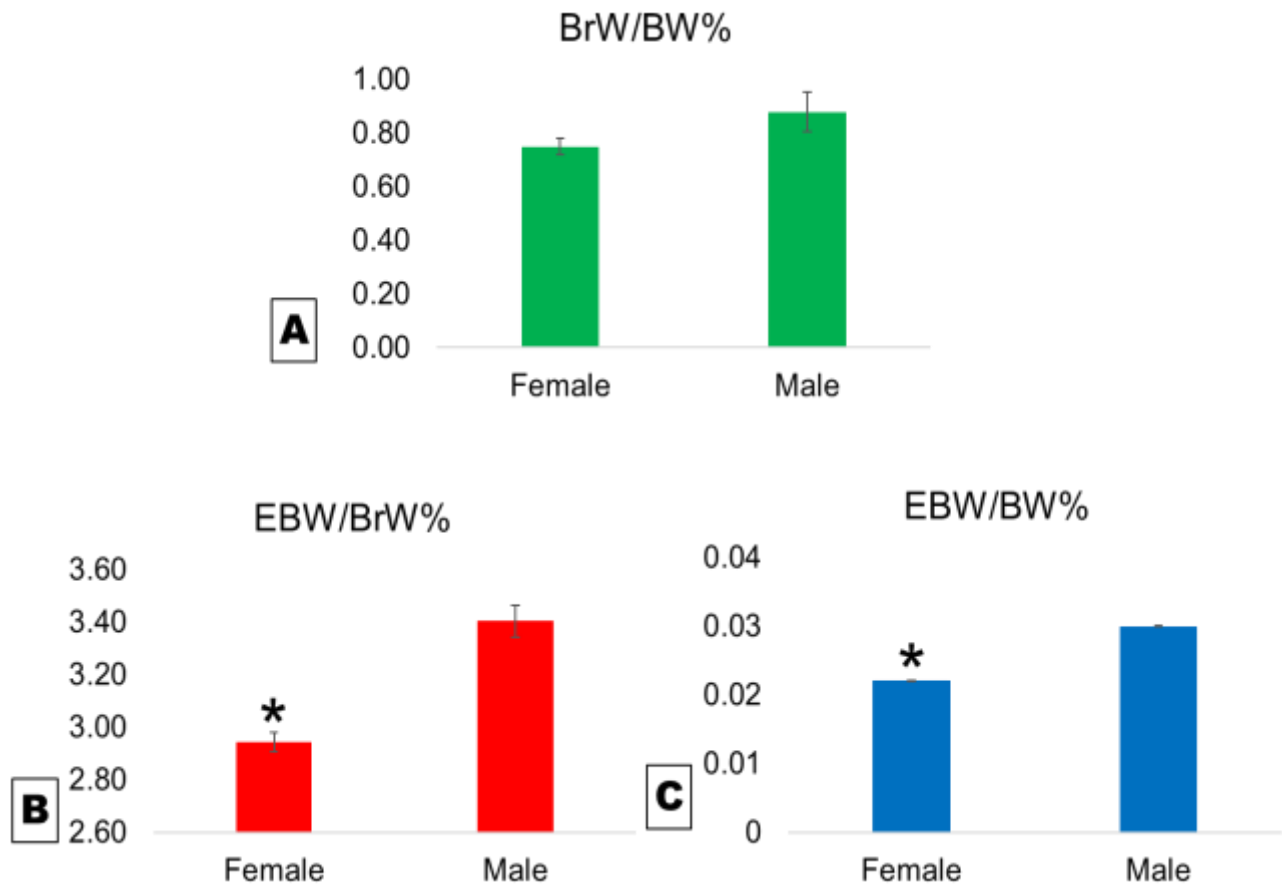


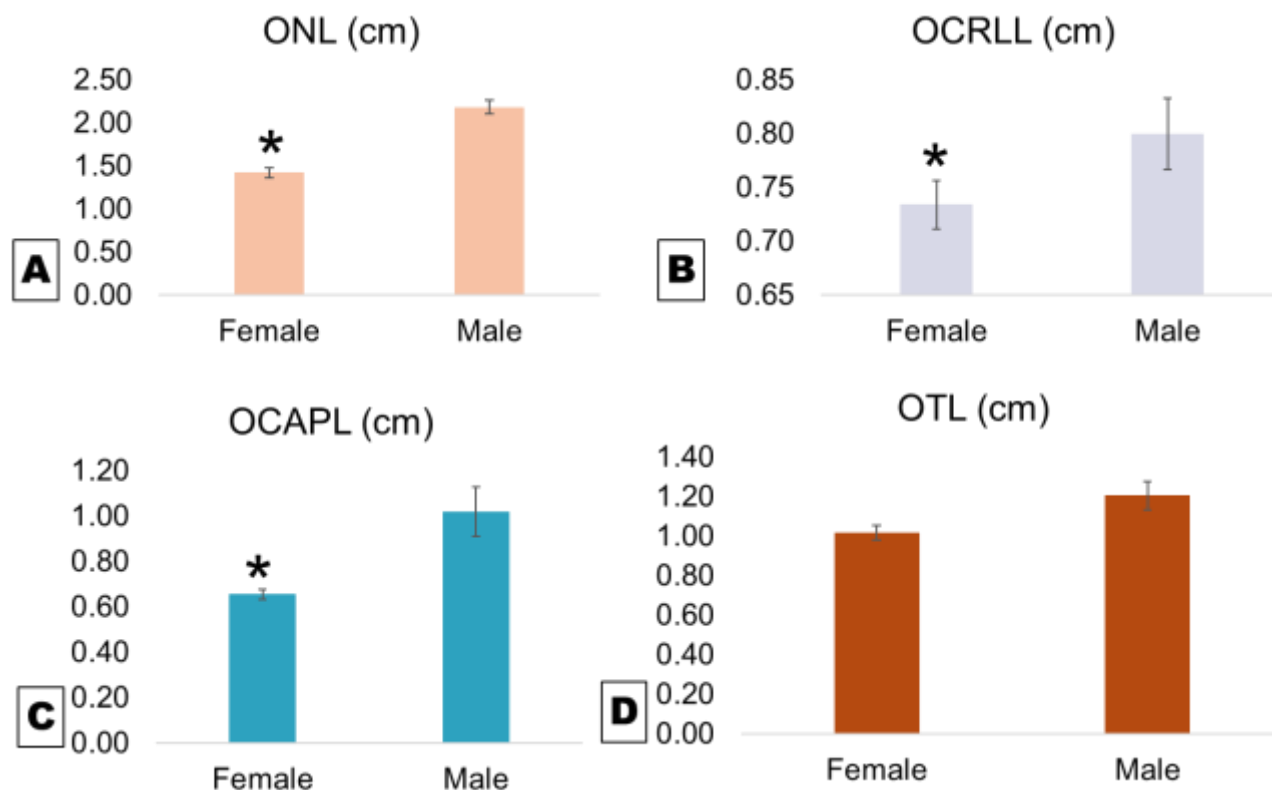
Figure 5: Sex differences in eyeball-brain-body weight indices of AGRs

EB = Eyeball; BrW = Brain Weight; BW = Body Weight; n= 5; mean  $\pm$  SEM; Unpaired *t*-test:  $p > 0.05$  (no significant difference)

Table 1: Sex differences in eyeball dimensions of AGRs

Variables	Female	Male	t	p
EL <sub>R</sub> (mm)	6.42 $\pm$ 0.14	7.06 $\pm$ 0.17	-2.887	0.020
EL <sub>L</sub> (mm)	6.70 $\pm$ 0.19	7.33 $\pm$ 0.30	-1.806	0.108
ED <sub>R</sub> (mm)	5.05 $\pm$ 0.15	6.17 $\pm$ 0.13	-5.674	0.000
ED <sub>L</sub> (mm)	5.48 $\pm$ 0.23	6.06 $\pm$ 0.09	-2.317	0.066
EW <sub>R</sub> (g)	0.20 $\pm$ 0.01	0.23 $\pm$ 0.01	-1.949	0.087
EW <sub>L</sub> (g)	0.20 $\pm$ 0.01	0.22 $\pm$ 0.01	-1.859	0.100

n=5; Unpaired *t*-test. EL<sub>R</sub>= Eyeball AP diameter right; EL<sub>L</sub>= Eyeball AP diameter left; ED<sub>R</sub>= Eyeball R-L diameter right; ED<sub>L</sub>= Eyeball R-L diameter left; EW<sub>R</sub>= Eyeball weight right; EW<sub>L</sub>= Eyeball weight left. Red text indicates significant difference between males and females.



**Figure 6: Comparison of dimensions of the Optic Nerve, Optic Tract, Optic Chiasma of AGRs.**

ONL = Optic Nerve Length; OCRL = Optic Chiasma Right-Left Length; OCAPL = Optic Chiasma Anterior-Posterior Length; OTL = Optic Tract Length.  $n = 5$ ; mean  $\pm$  SEM; Unpaired  $t$ -test:  $p > 0.05$  (no significant difference)

## DISCUSSION

In this study, visual pathway structures of African Giant Rats were described using morphological and microscopic approaches.

The observed absolute mean body weight of AGRs > 500 g for adults is in line with values ( $1010.00 \pm 25.10$  g) reported by Olude *et al.* (2015). Comparative studies have reported the mean weight values for smaller rodents including murines to be lower than the mean absolute body weight values for larger rodents like *Thryonomys swinderianus* (grasscutter); > 2 kg and porcupine; > 7 kg (Fournier and Thomas, 1997). The female AGRs revealed higher average absolute body weight than their male counterpart. This finding suggests a possibility of sexual dimorphism in the species; although differences were not significant in this study. Hergenroeder and Klish (1990) reported sex differences in the absolute body weight of a rodent species, *Mus musculus* (Mouse), attributing higher body weight values in females to accumulation of body fat due to their sedentary life style spending most of their time in burrows taking care pups, while the males are more active in search of food (Nowak, 1997; Perry, 2009).

The milky coloration of the AGRs' brain is in agreement with reported brain coloration for rodent species (Dwarika, 2008; Ibegbu *et al.*, 2014; Musa *et al.*, 2016). This coloration is a common manifestation of structures of the central nervous system linked with the presence of an integral biochemical component, lipid moieties (Aschner and Toews, 2010; Poon *et al.*, 2018). The oval-shaped brain of AGR is in line with reports by Ibe *et al.* (2014). The spherical-shaped eyeball of the AGR is in line with the morphology reported for rodents (Remtulla and Hallett, 2004; Jonathan *et al.*, 2005). This characteristic shape provides a wider field of view that provides the rodent the adaptive advantage to their nocturnal lifestyle (Schittny *et al.*, 2021). Monavarfeshani *et al.* (2017) in his review on the visual system of rodents revealed that the optic nerve exits the eye and projects ventrally to the optic chiasm in species like mice and rats. A similar trend is seen in the arrangement of the visual structures of AGRs.

Longitudinal fissure separating the two cerebral hemispheres and, a ventrally located brain stem with colliculi situated at the mid brain- tectum as observed in this rodent species is in line with reported brain morphology of rodents and other

mammalian species (Musa *et al.*, 2016; Ibe *et al.*, 2017; Pardo *et al.*, 2020). Morphologic features of the ventral surfaces including brain stem observed in this rodent species is in line with reported characteristics in rodents and other mammalian species (Olude *et al.*, 2017; Ibe *et al.*, 2021).

The mean brain weight of the adult AGR (> 5 g) is in agreement with reported values by Olude *et al.* (2016) (5.60 ± 0.06 g). This value is comparatively higher than that of the adult guinea pig (4 g), but less than that reported for adult rabbit (10.00 g), squirrel (7.6 g), marmoset (7.00 g) and porcupine (25 g) (Nzalak *et al.*, 2005; Eric 2006). Sexual differences in the brain weight observed in AGR is in line with reported trend in large rodent species like adult greater cane rat (> 8 g for males and > 9 g for females) (Byanet *et al.*, 2009).

The organosomatic index, in this case, the brain-body weight ratio is a metric that quantifies the percentage of brain mass relative to the absolute body weight of a species (Ibe *et al.*, 2017). In this study, the male AGR revealed higher values for brain-body weight ratio compared to their female counterparts. This finding is in line with reported higher values for brain-body weight ratio in other rodent species including mice and rats (Russell and Bulimia, 1979; Olude *et al.*, 2016; Agbon *et al.*, 2021). Higher values for brain-body weight ratio have been associated with intelligence in mammalian species as larger relative brain weight provides for more complex cognitive tasks, including behavioral flexibility, social interactions, and survival advantage in novel environments (Russell and Bulimia, 1979; Sol *et al.*, 2008; Roth and Dicke, 2005; Yu *et al.*, 2014). Thus, findings are suggestive of the male AGRs as more intelligent species compared to their female counter part and could be beneficial as an animal model for neuroscience related researches (Pallav, 2013; Edobor *et al.*, 2021; Genzel, 2021).

The mean eyeball weight for AGRs in this study was ≥ 0.2 g which agrees with reported values in AGRs (Olude *et al.*, 2011) (0.16 ± 0.01 g) and in other rodent species including *Notomys alexis* (Australian hopping mouse); 0.27 g (Smith, 1976) and *Cavia porcellus* (guinea pigs); 0.99 g (Latimer, 1951), but greater than the values reported for different strains of mice; 0.014-0.024 g (Zhou and Williams, 1999). The observed eyeball dimensions for the AGR were smaller than that of African grasscutter and humans with axial eye diameters of > 8mm (Peter-Ajuzie *et al.*, 2019) and > 20 mm (Augusteyn *et al.*, 2012) respectively. Organosomatic and organ-to-organ indices describes the ratio of organs to body weight and organs to certain organs. These indices are pointers to different evolutionary trends associated with ecological niches in certain environmental changes. This tells how each species has adapted over time (Schmidt-Nielsen, 1984, Ronald and Bruce, 1990).

Differences in the size of the left and right bodily parts have been observed in various mammalian species (van der Meulen *et al.*, 2005). Observed differences in the measured values (weight and dimensions) of the AGR eyeball is

suggestive of biological asymmetry. Findings are in line with reports on asymmetric characteristics of brain parts in rodents and other mammals (Ocklenburg and Güntürkün, 2018).

The dimensional differences in eyeballs values for AP and R-L diameter between males and females is suggestive of sexual dimorphism. This finding agrees with reported values by Olude *et al.* (2011) who reported that the antero-posterior (AP) eyeball circumference and the right mediolateral (R-L) eyeball circumference of the right and left eyeballs were greater in males than in females but this did not translate into weight as the eyeball weights between the sexes for the left and right eyeballs were similar.

The AGR eyeball indices (eyeball-body weight and eyeball-brain weight ratios) showed significant sexual differences with males having higher values than their female counterparts. This finding is in agreement with sexual dimorphic characteristics in the eyeball indices reported for rodents, mice, rats, and gerbils (Breedlove and Hampson, 2002; Ebrahim *et al.*, 2018). Graw (2010) reported that male rodents often have larger eyeball indices compared to females of the same species. Larger eyeball indices in male rodents have been associated to varying behavioral patterns and ecological roles. The males are habitually engaged in more exploratory, and territorial behaviors (Kiltie, 2000; Breedlove and Hampson, 2002; Ebrahim *et al.*, 2018).

Dimensions of the male AGR optic nerve and chiasma revealed significant difference when compared to their female counterparts. This finding is in line with reports on the diameter of AGRs' structures of the visual pathway including optic nerve, chiasma and tract (Jeffery and Erskine, 2005). Similar trend was observed in other rodent species including mice and rats (Kaas, 2008; Chuang *et al.*, 2021). This suggests that males often exhibit greater sexual differences in various aspects of the visual system which may be related to differences in visual processing, spatial perception and other sex-specific behavioral and ecological requirements (Kaas, 2008; Chuang *et al.*, 2021).

**Conclusion:** There exists sexual dimorphism in the morphometric parameters of visual pathway structures in the AGR. Most of the assessed morphometric values were higher in males. These differences are suggestive of evolutionary advantage in males compared to females as they go out in search for food and easy escape from predators within their natural habitat.

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