COMPARATIVE ANATOMY OF OLFACTORY BULBS IN AFRICAN GIANT POUCHED RATS *(Cricetomysgambianus, Waterhouse)* AND WISTAR RATS.

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AUGUST, 2015

DECLARATION

I declare that the work in this thesis titled **"Comparative Anatomy of Olfactory Bulbs in African Giant Pouched Rats (*Cricetomysgambianus*, Waterhouse) and WistarRats"**hasbeen performed by me in the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria, Nigeria under the supervision of Prof. S.A. Ojo andProf. B.I Onyeanusi.The information derived from various literatures has been duly acknowledged in the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other University.

David Pindar BUKAR	----------	----------
Name of Student	Signature	Date

CERTIFICATION

This thesis titled **"COMPARATIVE ANATOMY OF OLFACTORY BULBS IN AFRICAN GIANT POUCHED RATS (*Cricetomysgambianus*, Waterhouse) AND WISTAR RATS"** by David Pindar Bukar meets the regulations governing the award of the degree of Masters ofScience in Ahmadu Bello University, Zaria, is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Thesis is dedicated to my Son David Junior Pindar, my Lovely wife Mrs Charity David and the entire Pindar's family.

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I give God almighty all the Glory for giving me the ability to carry on with this thesis up to this stage. I also want to cease this opportunity to express my profound gratitude and deep regards to the Chairman of my Supervisory Committee Prof. S.A. Ojoforhis exemplary guidance, mentoring and constant encouragement throughout the course of this thesis. The support, mentoring and guidance given by him will go a long way to help me in my professional journey in life.

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ABSTRACT

Comparative anatomy of olfactory bulbs of African Giant Rats (AGR) (*Cricetomysgambianus,*Waterhouse) and Wistar Rats (WR) was carried out using, twenty four adult (24) adult AGRs comprising twelve (12) females and males respectively, and twenty four (24) adult WRs comprising twelve (12) females and males respectively for this study. The AGRs and WRs were euthanized under light anaesthesia using gaseous chloroform in a confined glass container. After fixing the heads of the two rats in Bouins solution, the brain was carefully extracted, weighed and recorded. The olfactory bulb was then secured from the brain, weighed and recorded. Gross anatomical features of the brain and olfactory bulbs were examined from the dorsal, ventral and sagittal views taking into consideration their shapes, sizes, surfaces, and attachments. Furthermore, morphometric study of the brain and olfactory bulbs of the AGRs and WRs were conducted by measuring weight, length, height and the volume as morphometric parameters. The brain and olfactory bulbs were fixed in Bouin's solution, dehydrated in graded alcohol for 24 hours; the Bouin's solution was cleared using xylene for 2 hours. Routine paraffin embedding method for histological sectioning of the samples was used. Hematoxylin and Eosin, Cresyl Fast Violet and Enarson's staining methods were used toview and compare the individual cells types, cell layers and the cell organization of the olfactory bulbs of the female and male AGRs and WRs. Slides were viewed under light microscopy at various magnifications, while photomicrographs of olfactory bulbs and their individual cell types, cell layers and cell organization were taken using digital camera. This study has established a comparative base-line data for the brain and olfactory bulbs of both female and male AGRs and WRs giving a detailed description of the neuroanatomical characteristics with emphasis on the morphology, morphometry and histomorphology. The 3 stains gave a clear histology

of the concentric laminar organization of the olfactory bulbs showing 6 layers in both rats.Glomerular cell layer in AGR was 2 layers thick, while that of WR was 3 layers thick. Mitral cells were numerous and well distributed in the mitral cell layer of both rats. Among the 3 stains,cresyl fast violet gave a better histograph of the olfactory bulbs layers and cells. There will be need to carry out further studies to determine and evaluate the macromastic features of the cell types, cell layers and cell organization using immunohistochemistry, neurone tracing method and micrometry.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Research

The African giant pouched rat (*Cricetomysgambianus*-Waterhouse) belongs to the family Cricetidaeand the order Rodentia. Like dogs, giant African pouched rats have a highly sensitive sense of smell. Previous research has shown that the AGR, which are relatively large rodents (1-2 kg body weight) that can live for 7-8 years in captivity, can be readily trained through operant conditioning procedures to detect land mines (Poling *et al*., 2010 a, b; Poling *et al*., 2011); to detect the presence of *Mycobacterium tuberculosis*, the bacillus that causes tuberculosis, in human sputum (Mahoney *et al*., 2012; Poling *et al*., 2010c; Weetjens*et al*., 2009); and to detect salmonella bacteria in horse faeces (Mahoney *et al*., 2014). They are found in South Africa, in the region of Sahara desert and these include countries such as Nigeria, Ghana among others (Ajayi*et al*., 1978; Kingdon, 1989). The rats are called *Burugu* in Hausa, *Okete* in Yoruba and *Ewi* in Igbo language.

They are nocturnal and almost inactive during the day, but come out at night in search of food (Nowak, 1999). They inhabit a variety of habitats ranging from arid areas to temperate areas but need some form of shelter to survive (Ajayi, 1977a; Kingdon, 1989).During the day (inactive period) they live primarily in burrows constructed using their teeth and forepaws to loosen and their hind limbs to push evacuated soil away (Ewer, 1967).

The African giant rats have soft, grey coats with white fur on the belly. Their long tails are scaly and they have narrow heads. The main physical characteristics of the *Cricetomys* in general are their large cheek pouches. These pouches can expand to a great size, allowing African giant rats to transport massive quantities of food, if

necessary (Ajayi, 1977b; Kingdon, 1989; Ryan, 1989; Nowak, 1997). Their ears are round, naked and large while the eyes are small (Morris, 1963; Ajayi, 1975; Skinner, 1990). These rats have been found to be fairly tame though and docile in captivity even though shortly after being captured, they tend to exhibit escape reactions such as violent struggles by scratching with their nails and hitting their heads and tail against their cages (Oke, 2009).

African giant rats are hoarders and omnivores (Kingdon, 1989; Ajayi, 1977a; Nowak, 1997; Amador, 2003). The rats are relatively inexpensive to transport, house, maintain and are not dependent on a single human handler to work well, and maintain accurate performance during long periods of repetitive work. In recent years, the possibility of using scent-detecting dogs and rats to detect disease has drawn considerable interest (Moser and McCollough, 2010; Wells, 2012). There is evidence that African giant rat may be a useful supplement to the global effort to control illicit cigarette trade (Mahoney *et* al., 2014).In Nigeria, African giant rat serves as supplementary source of protein diet to rural dwellers. The olfactory system has also long been an attractive model to study cellular mechanisms underlying the encoding, transfer, processing, and decoding of sensory information (Ennis *et al*., 2007; Ngwenya*et al*., 2011).Studies have been done on the central nervous system of African giant rat (Nzalak, 2005; Ibe*et al*., 2011) but to the best of my knowledge, there is no work done on the comparative anatomy of the olfactory bulbs of African giant rats and other rodents.

The Wistar rat is a species of rat that is as common as the African giant rat in this part of the world. Wistar rats, also called laboratory rats because of their small size, are not readily sought for as food delicacy by the local populace (Onyeanusi*et al*., 2009).Wistar rats are albino and outbred, they originate from stock established in the Wistar Institute (Philadelphia, PA, USA) in the early 1900's. Rats from Wistar Institute were distributed

globally and now there are many stocks available from different breeders in different countries. It is well known that Wistar rats from different breeders have different characteristics, although they are still called by the common stock name "Wistar" (Sasaki *et al*., 2006; Yamada *et al*., 1979). Wistar rats show rapid growth, good temperament, relatively small body size and high survival rate in long term experiments. They are used widely in toxicological, carcinogenic, pharmacological and general biomedical studies (Maekawa*et al.,* 1983; Watanabe *et al.,* 1997). Rat role as vectors of odor molecules and respiratory dynamics have been considered as an integral part of olfactory percept in both animals and humans by various author (Johnson *et al.,* 2003; Kepecs*et al.,* 2006; Mainland and Sobel, 2006). It has been shown that rats adapt their sniffing strategy, both in frequency and flow rate, when performing odor discrimination and detection tasks (Youngentob*et al*., 1987). Therefore, the question of how these sampling variations affect olfactory activities is of growing interest.Studies have been done on the biology of this rat in Nigeria.

The olfactory bulb is an oval enlargement in front of the pole of the hemisphere. It's convex superficial face fits into the ethmoidal fossa and receives numerous olfactory nerve fibers through the cribriform plate (Ojo*et al*.,1987).

The olfactory system consists of two parallel systems: the main olfactory system and the accessory olfactory system. The sense of olfaction is critically important for food consumption, emotional responses, aggression, maternal and reproductive functions, neuroendocrine regulation, and recognition of conspecifics, predator and prey (Ennis *et* al., 2007). In many species, olfaction plays a more pivotal role in these functions than in humans, with olfactory cues exceeding visual or auditory cues in importance (Ennis *et* al., 2007). In all vertebrates, the main olfactory system detects and processes the vast

majority of chemical cues that enter the nasal cavity. This system is a chemical categorizer, first detecting a chemical and then cataloguing it in the cortex for later recall. The role of the main olfactory system in the detection and processing of chemosensory cues that trigger innate, hard-wired physiological or behavioural responses is still somewhat controversial but has recently received considerable attention(Restrepo *et al*., 2004; Shepherd, 2006; Kobayakawa*et al.*, 2007). Sensing an odour begins with the Olfactory Receptor Neurones (ORN) located on the nasal epithelium (Wilson and Mainen, 2006). The axons of the stimulated ORN transmit the sensory signal to approximately two locations in a single olfactory bulb known as glomeruli, which are simply the synapses joining the ORN axons with the dendrites of mitral/tufted (M/T) cells, and transfer the signal to the olfactory cortex (Mombaerts*et al.,* 1996; Albeanu, 2008). Mitral/Tufted cells also communicate with each other directly and indirectly. M/T cells form dendrodendritic synapses with granule cells in the external plexiform layer, and can inhibit other M/T cells through granule cells (Wilson and Mainen, 2006). M/T cells may also directly influence their neighbours through a process known as spill-over.

1.2Statement of Research Problem

African Giant Pouched rat is a common animal in sub-African region. The Nigerian Wildlife Conservation Committee has encouraged its domestication effort, through breeding for the purpose of conservation and supplementation of dietary meat supply (Ikede and Ajayi, 1976), but this is yet to come to actualization.

Effort is still on the way to make this animal a pet. This has not been completely achieved because some remain aggressive and destructive (Isaksen, 1997). Plan is still on the way to classify this rodent as laboratory model for research (Ajayi, 1975;

Olayemi and Adeshina, 2002). All the above shortcomings are because the biology and behavior of this animal are not fully understood.

One of the fundamental concerns of neuroscience is to understand how the brain perceives and processes the outside world. Olfaction is one of the ways we sense the world, yet the study of the olfactory system has many unanswered questions. African giant pouched rat is a highly macro-mastic animal with unique sniffing capabilities, but there are no comprehensive and comparative works carried out on the anatomy of the olfactory bulbs of this rat and other rodents to mark possible anatomical difference(s).

Some years back the World Bank provided a grant of US$ 160,000 to build extra laboratories in Tanzania to carry out more clinical trials and train up to 400 Tuberculosis Sniffing African giant rats (Daniel, 2004). Tuberculosis (TB) is a growing worldwide epidemic. It is imperative to detect TB as early as possible. With early detection, good health care facilities and access to drugs, TB is curable. Preliminary tests suggest the rats could test as many as 150 saliva samples for TB in just 30 minutes. By contrast, human technicians using a microscope can test only 20 samples a day (Maggie, 2003). Comparative studies of the morphology, morphometry and histomorphology of the cells, cellular layers and organizations of olfactory bulbs in African Giant Pouched Rat and Wistar rat, may give a better reason anatomically why African Giant Pouched Rat has great sniffing capabilities, hence a potential tool for diagnosis of TB and detection of landmines.

1.3Justification of Research

The sniffing capabilities of African Giant Rat serve as a quick, effective, cheap and alternative way to diagnose TB especially in the African sub region. Comparative studies of morphology, morphometry and histomorphology of the cells, cellular layers and organization of olfactory bulbs of the two rats may give an anatomical clueon why the African Giant rat has unique sniffing capabilities to identify distinct smell of chemicals in infected sputum samples and detection of landmines.

The results of the current study may shed more light on our understanding of the neuroanatomy of the African Giant rat and will add to the body of information cumulating on African Giant Pouched Rat (Nzalak, 2002;Nzalak*et al*., 2008;Ibe*et al*., 2011). This will help in understanding their neurobiology and behaviour hence the ease for domestication.

1.4Aim of the Study

The aim of the study is to carry out a comparative evaluation of the morphology, morphometry and histomorphology of the cells, cellular layers and organization of the olfactory bulbs in African Giant rats and Wistar rats.

1.5Specific Objectives

i. To study thecomparative morphology and morphometry of olfactory bulbs in African giant rats and Wistar rats.

ii. To study thecomparativehistomorphology of the olfactory bulbs in African giant rats and Wistar rats.

iii. To describe and correlate the cellular layers and organizations of the olfactory bulbs in the African giant rats and Wistar rats.

iv. Attempt to relate their morphology and cellular layers and organizations with their functions.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1The Brain

The brain (encephalon) is the centre of the nervous system in all vertebrate animals. It is located in the head, usually close to the primary sensory organs such senses as visions, hearing, balance, taste and smell. The brain of a vertebrate is the most complex organ of its body (Pelvig*et al*., 2008).

It is contained within the cranium and constitutes the upper, greatly expanded part of the Central Nervous System (CNS) (William and Warwick, 1989).Both the spinal cord and the brain are covered in three continuous sheets of connective tissue, the meninges. From outward-inward these are the dura mater, the arachnoid and the pia mater in mammals (Millen, 2003).The brain is constantly bathed by lymph-like fluid called the cerebrospinal fluid (CSF) which fills the central duct system and spaces between the meninges and the brain. The brain is important not only for storage and processing of information but also for the release of enzymes, hormones and neurotransmitter substances (Cazalis*et al*., 1985).

The brain and spinal cord make up the central nervous system (CNS). Some regions of the CNS appear white (white matter) while others have grayappearance (gray matter). The white matter is formed by dense accumulation of nerve fibres individually enveloped by myelin, white lipid protein insulation. The gray matter lacks myelin accumulation, but is rich in cell perikaryon. The CNS is built up in a supporting framework of connective tissue cells known as the neuroglia (David, 1975). These neuroglial cells (astrocytes, oligodendrocytes, microglia and Ependymal cells) are involved in the transmission of messages (Fletcher, 2006).

Glial cell are said to be the most numerous cell type in the brain (Doetsch, 2003; Nishiyama*et al*., 2005). The ratio between the total number of glial and neuronal cells (glial/ neuron ratio) in the cerebral cortex has been shown to increase with brain size (Stolzenburg*et al*., 1989; Hawkins and Olszewski, 1957).

The average brain weight of various species has been documented. These include rabbit, 10-30g; porcupine, 25g; dog (beagle), 72g; rat (400g body weight), 2g; guinea pig, 49g; Squirrel, 7.6g;andgrass cutter 10.5g (Byanet*et al*., 2008). The brain weight of African giant rat was 4.94g (Nzalak, 2008). Male brain is about 10% larger than that of female brain and weighs 11-12% more than that of female. Male's heads are also about 2% bigger than female's. This is due to larger physical stature of male. Males' largermuscle mass and larger body size requires more neurons to control them. The brain weight is related to the body weight partly because it increases with increasing height (Ho*et al.*, 1980).

2.2Olfactory Bulbs

The olfactory system consists of two parallel systems: the main olfactory system and the accessory olfactory system. The sense of olfaction is critically important for food consumption, emotional responses, aggression, maternal and reproductive functions, neuroendocrine regulation, and recognition of conspecifics, predators, and prey (Ennis *et al*., 2007). In many species, olfaction plays a more pivotal role in these functions than in humans, with olfactory cues exceeding visual or auditory cues in importance (Ennis *et al*., 2007). The olfactory system has also long been an attractive model to study cellular mechanism underlying the encoding, transfer, processing, and decoding of sensory information(Ennis *et al*., 2007; Ngwenya*et al*., 2011).

In rodents, the olfactory system appears to be the most imperative for the perception of chemical signals that allows the organism get the information from the environment (Eduardo *et al*., 2012). Olfaction starts with the direct interaction of odorant molecule with the olfactory receptors of the sensory neurons that send their axons up to the olfactory bulb, step that constitutes the first relay of the olfactory information to the central nervous system. Olfactory bulb has been divided into two different but complementary systems. They differ in their anatomy, projection and function. Sensory receptors of the main olfactory sensory neurons (OSNs) sited in the main olfactory epithelium and projecting into the main olfactory bulb (MOB). For the accessory olfactory system, the receptors are located in the vomeronasal organ and project to the accessory olfactory bulb (AOB). Both MOB and AOB are an interface between the OSNs and higher centres, and their position in olfaction is often compared to the thalamus in other sensory systems (Shepherd, 2005). These two anatomically distinct olfactory systems were described as functionally distinct, with the main olfactory epithelium detecting volatile odorants and the vomeronasal organs detecting non-

volatile pheromones through direct physical contact with source (Mori *et al*., 2001). The Main olfactory bulb in rodents is situated at the rostral pole of the cranial cavity and is connected to the frontal cortex by a slender peduncle. The bulb can be described as an elongated onion that composed of distinct layers or laminae that are organized as concentric circles, and the organization and synaptic connectivity have been well documented in a number of rodents and other vertebrate species (Halasz and Shepherd 1983; Kratskin and Belluzzi, 2003; Kosaka and Kosaka, 2009) and in African Elephant (Ngwenya*et* al., 2011). (Jenkins, 1978) described the olfactory bulb in the dog as rounded ventro-rostral projection which lies in the cribriform fossa of the ethmoid bone. He further explained that inside the olfactory bulb, there is a cavity which connects it with lateral ventricle by way of an olfactory stem. This olfactory stem may be occluded, he added. According to (Carina *et al*., 1998) the dog's olfactory bulb is larger than that of man and the armadillo's olfactory bulb accounts for 30% of the total length of the brain. In fishes, the olfactory bulbs are as prominent as the cerebral hemispheres, reflecting the importance of olfaction to their survival (George, 1973).

The olfactory bulbs (OBs), which are usually a pair of independent structures in vertebrates, are anatomically fused in crows and sparrows, forming a very unique morphological feature (Crosby and Humprey, 1939).It has long been known that the OBs are fused in the American crow; the raven (*Corvuscorax*), which belongs to the same family as the crow; and Passeriformes species such as the sparrow (Crosby and Humphrey, 1939; Bang, 1971). This fusion was observed using both gross anatomical and histological examinations. However, research on olfaction has rapidly progressed after the discovery of olfactory receptors by (Buck and Axel, 1991).

The presence of accessory olfactory bulb has been established (Shepherd and Haberly, 1970; Chuah and Zheng, 1987; Price, 2004). An accessory olfactory bulb is present in

lower tetrapods that have a largevomeronasal organ (George, 1973). In Marsupials, the OBs are large, ovoid and gray structure that project into the neopallium. (Muller and O'Rahilly, 2004) described the accessory olfactory system to be more marked in rodents and monkeys than in humans.

2.2.1 Embryology of olfactory bulb

In lower vertebrates, adult neurogenesis continually supplies additional neurons capable of regenerating entire brain tissues. In contrast, neurogenesis is restricted to a small number of regions of the adult mammalian brain, in which it generates neurons mainly for replacement purposes. The generation of new neurons is sustained throughout adulthood in the mammalian brain due to the proliferation and differentiation of adult neural stem cells (Zhao *et al*., 2008). Two forebrain structures actively demonstrate adult neurogenesis namely, the subventricular zone of the lateral ventricles (SVZ) and subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus (Ihunwo and Pillay, 2007). The cells generated in the SVZ migrate tangentially along the rostral migratory streams (RMS) toward the olfactory bulb (OB) (Altman and Das, 1965; Luskin, 1993; Gage, 2000; Alvarez-Buylla and Garcia-Verdugo, 2002). Tens of thousands of new cells are generated and reach the mammalian olfactory bulb (OB) each day (Alvarez-Buyllaand Lim, 2004). However, only a small percentage of these cells persist long enough to differentiate into mature interneurons. Previous studies have shown that only 50% of the new neurons migrating into the bulb survive for longer than a month after their initial generation and differentiate principallyinto granule and periglomerular cells (Petreanu and Alvarez- Buylla, 2002; Yamaguchi and Mori, 2005; Alonso *et al*., 2006; Moure*t et al*., 2008**). (**Altman and Das, 1965) provided the first evidence of newborn neurons in the adult rodent brain about half a century ago. Since then, persistent neuronal generation has been unequivocally demonstrated in virtually all

mammals (Alvarez-Buylla and Lim, 2004; Ming and Song, 2005; Zhao *et al*., 2008), including humans (Eriksson *et al*., 1998). Neural stem cells in the subventricular zone (SVZ) and rostral migratory stream (RMS) continue to produce neuroblasts throughout life. These neuroblasts migrate to the olfactory bulb (OB), where they differentiate into two major classes of interneurons: the granule cells (GCs) and periglomerular cells (PGCs).

The olfactory epithelium (OE) has a unique capacity for continuous neurogenesis, extending axons to the olfactory bulb with the assistance of olfactory ensheathing cells (OECs). The OE and OECs have been believed to develop solely from the olfactory placode, while the neural crest (NC) cells have been believed to contribute only the underlying structural elements of the olfactory system (Hiroyuki*et al.*, 2011).

The sensory organs of the vertebrates head are derived from two embryological structures, the sensory placodes and the cranial neural crest (NC) which arises from the border between neural and non- neural ectoderms on the lateral edge of the neural plate. It contributes to the formation of the peripheral sensory nervous system in an intricate relationship during cranial development. Placodes are discrete areas of thickened non-neural epithelium that are formed in characteristic positions in the head of vertebrate embryos and give rise to the paired sensory organs, including the olfactory system. The NC is a multipotent population of migratory cells unique to thevertebrate's embryo that delaminate from the neural epithelium and migrate throughout the embryo to give rise to a wide variety of cell types (Le Douarin, 1999; Sellecke*t al.*, 1993).

The olfactory organ has been shown to arise from a combination of the olfactory palcode and cranial NC cells, with the olfactory placode giving rise to the olfactory sensory neurons and supporting cells of the olfactory epithelium (OE) (Klein and Graziadei, 1973; Mendoza *et al*., 1982; Couly and Le Douarin, 1985), and the

NCcontributing to the structural elements of the nose. The role of the olfactory placode in olfactory development was first experimentally demonstrated in the early twentieth century when resection of the olfactory placode in amphibians was shown to disrupt the development of the olfactory bulb (Burr, 1916). The importance of NC cells in the development of the olfactory system was demonstrated in rSeyrats with a mutation in the pax 6 gene in which impaired migration of midbrain crest cells into the frontonasal mass led to the loss of the nasal placode (Osumi-Yamashita*et al*., 1997) and retinoic acid signalling from NC cells was found to be necessary for olfactory placode development (Bhasin*et al.*, 2003). The convergence of placode and NC cells in the embryo to give rise to the olfactory organ has made uncovering the developmental origins of the olfactory components very complicated. The olfactory placode has been described as one of the most versatile placodes, being unique in its capacity to give rise to glial cells and stem cells capable of regenerating various differentiated cell types of the OE throughout life. Also the OE, with its capacity for continual neurogenesis in adult, has been a hotspot for the study of neuroscience (Hiroyuki*et al*., 2011).

Two theories have been proposed to explain the mechanism underlying the formation of olfactory bulb: The "Protomap" model and 'Protocortex' theories.

In the 'protomap' model, cortical regions are patterned prior to the migration of the newbornneurons (intrinsic control) (Rakic, 1988), an event presumably specified by important molecular determinants (Fukuchi-Shimogori, 2001). In this model, the arrival of innervating axons would merely serve to modify and refine the protomap (an important facet of maintenance).

The 'protocortex' theory predicts that the development of the olfactory bulb would be a secondary process; dependent upon the arrival of the olfactory sensory neuron fibres

from the olfactory epithelium. The formation of the olfactory bulb involves differentiation of several populations of cells and the initiation of central projections, all under the temporal and spatial pattern of gene expression. In summary, it has already been demonstrated that olfactory bulb cells can survive without the olfactory epithelium and can never begin to differentiatewithout the arrival of its primary afferent fibres. Hence the protomap has been proven to be the way in which olfactory bulb developed (Lopez-Mascaraque*et al.,* 1996).

Furthermore, olfactory sensory neurons are able to survive and are capable of completing their continuous replacement and axonal regeneration even in the absence of olfactory bulb, although the rate of replacement may be affected. Nevertheless, both structures seem to be independent of one another for their respective survival. Theobservations do not preclude the conclusion drawn by some authors that the olfactory sensory neuron axons influence later stages of olfactory development (Lopez-Mascaraque*et al*., 1996; Gong and Shipley, 1995; Treloar*et al*., 1997; and Matsutani *et al*., 2000).

The development of olfactory bulb in the foetal period has been described (Hinds, 1972) to involve elongation and rotation. The olfactory peduncle develops and the olfactory tract is present at about eleven weeks post-fertilization (Muller and O'Rahilly, 2004). The olfactory structures in staged human embryos were investigated in 303 human embryos by (Muller and O'Rahilly, 2004). Olfactory system in human is far from being rudimentary and indeed, almost all the major olfactory structures found in rats were also seen to be present in humans (Price, 2004). Adult neurogenesis depends on strain and age of rats and mice (Kempermann*et al*., 1997) with the African giant rat (AGR) showing increase in adult neurogenesis from neonates to adult (Olude*et al*., 2014). In the developing AGR examined, adult neurogenesis in the DG persisted across the age

groups, although it declined with increasing age; a feature commonly reported in all mammalian species (Kuhn *et al*., 1996; Eriksson *et al*., 1998; Maslov*et al*., 2004; Leuner*et al*., 2007; Grabiec*et al*., 2009). If the hypothesis that adult neurogenesis plays a role in spatial memory and cognition is anything to go by, juvenile AGR have commendable capacity for spatial memory and behaviour perhaps justifying their use in landmine detection and tuberculosis diagnosis as neurogenesis was confirmed in the olfactory bulb with Doublecortin (DCX) staining at all levels of the olfactory cells (Olude*et al*., 2014). Also the presence of positive cells in the piriform and somatosensory cottices of the AGR may be linked to the behavioural and functional aspects of the AGR in landmines and tuberculosis diagnosis (Verhagen*et al*., 2003; Weetjens*et al*., 2009).

2.2.2 Gross and morphometric organisation of the vertebrate olfactory bulb

On the anterior extremity of the cerebrum, the olfactory bulb sits on a flat band of white nerve fibres named olfactory tract, which continues posteriorly as the medial and lateral striae (Sisson and Grossman, 1953), the latter is delineated from the underlying lateral cerebral gyri by the sulcus rhinalis. At the angle of divergence of the striae occurs the olfactory trigone, whose separation from the posterior positioned piriform lobe is by the lateral fossa (Kavoi and Jameela, 2011).Parameters of specific parts of brain show differences that reflect functional requirements between and within species (Kruska, 1988; Kaas and Collins, 2001). Earlier work on olfactory brain morphometry focuses mainly on the bulb asymmetry (Heine and Galaburda, 1986; Hirano *et al*., 1989) and sexual dimorphism (Byanet*et al*., 1989) within individuals' species, allometry within genera (Gittleman, 1991) and the effects of disease on the bulb size (Turetsky*et al*., 2000; Turetsky*et al*., 2003). Furthermore, changes in parameters of certain brain parts

have been attributed either to an increase in the number of neurons and or their connectivity (Kaas, 2000).

Morphometry is the scientific study of the dimensions of gross anatomical structures of organisms, with particular reference to their linear measurements and weights. These had often been done by direct measurements on formalin fixed specimens especially on the brain. Results have varied and accuracies are still being discussed (Peters *et al*., 1998; 2000). Although the magnetic Resonance Imaging technique pronounces to yield more accurate and acceptable results, it is expensive and still not universally available.

It is well known that laterization of function and structure is a feature of the human brain as well as that of other species, including birds and other vertebrates (Kolb *et al*., 1982; Mayhew*et al*., 1996a; 1996b). However, it is not clear if these functional differences are reflected in morphologic or morphometric differences. Study on the cat brain with respect to its weight, size and thickness of cell strata of the olfactory bulb, demonstrated a significant correlation between the brain weight and the body weight but olfactory brain size did not correlate significantly with the body weight (Hirano *et al*., 1989).

Like cerebral right – left asymmetry reported in rat, mouse, rabbit and Cat (Kolbe *et al.*, 1983; Sherman *et al*., 1982), olfactory asymmetry has been shown in the brain of rat (Heine and Galaburba, 1986; Khanna and Sengupta, 2001), cat (Hirano *et al*., 1989), and olfactory hypoplasia in Prokr2 null mice (Haydn et al., 2007). In humans, MRI-based OB asymmetry has been linked to schizophrenia (Hirano *et al*., 1989), and Parkinson's disease (Turetsky*et al*., 2003) and septo-optic dyspasia (Herting*et al*., 2008). Recently OB enlargement after rhinosinusitis treatment (Gudziol*et al*., 2009) has been reported.

The greatest width(diameter) of olfactory bulb and also the ratio of the bulb width to that of corresponding cerebral hemisphere increase from humans to goat, to dog. This seems to match with reports on birds (Bang and Cobb, 1968) where olfactory bulb to hemisphere width ratios were 4% in the house sparrow, 15% in the domestic fowl and 28.7% in the turkey vulture (a carnivorous bird). These proportional differences were attributed to the varied degrees of olfactory dependency on feeding in the birds. In birds, the olfactory ability is reflected by the Olfactory Bulb Brain Ratio(OBBR), which is ratio of the size of the OB to that of the cerebral hemisphere (Bang and Cobb, 1968; Bang, 1971). For instance, the OBBR of brown kiwis (A. autralis), which has the most developed olfactory sense of all birds, is 34.0, whereas the OBBRs of canaries and sparrows, in which olfaction has not yet been documented, are 6.0 and 4.0 (Bang, 1971).

Individual length measurement of the olfactory bulb and also the OB together with the tract and stria gives the highest values in the dog followed by human, and the lowest value in the goat. In the dog, the long olfactory bulb tract and stria may have resulted from the characteristic narrowing and lengthening of the cerebral hemispheres at the level of the anterior poles (Sisson and Grossman, 1953). In humans, it is likely that the exceptionally large frontal lobes (Cavinesset *al*., 1996) over lie these structures to make it fairly long.

2.2.3. Histological organization of vertebrate olfactory bulbs

The main structure of the olfactory bulb includes olfactory nerve layer, olfactory glomerular layer, external plexiform layer, mitral layer, internal plexiform layer and granule cell layer (Zhu, 2002).These features of the olfactory bulb's structure make it easier for information processing, and they also provide a structural foundation for the spatial encoding of olfactory information. The olfactory receptors are ciliated epithelial

cells with an array of receptors capable of detecting thousands of different odors (Diana *et al.,* 2000). The receptor neurons themselves do not project to the cerebral hemispheres. Their axons project up through the cribriform plate of the skull to synapse on the dendrites of the mitral cells of the olfactory bulb (Diana *et al*., 2000)

According to classical research, the olfactory bulb includes four kinds of cells: Mitral cell, granule cell, tufted cell and short axon cell (Colonnier, 1968). Of these neurons, the granule cell has long been known to be morphologically unusual having no typical axonand recent electron microscopic studies have shown that it participates in unusual reciprocalsynaptic connections with the mitral cells (Hirata, 1964; Andres, 1970; Rall*et al*., 1966).

Mitral cells are the largest cells in the olfactory bulb, and they are also the major efferent neurons of the olfactory bulb as indicated by light microscopy and electron microscopy (Andres, 1970) studies.The large mitral cells are the most characteristic element, and their cell bodies are closely packed together forming a well-defined layer (Allison, 1954). The dendrites of mitral cells can be classified into primary and secondary dendrites, and both of the smooth primary and secondary dendrites pass superficially into external plexiform layer, but only the primary dendrites project down to the olfactory glomerulars (Mori, *et al*., 1983; Orona., *et al*., 1984). Within the glomerular the mitral cell dendrites are in synaptic contact with the olfactory nerves and also with the periglomerular cells, but elsewhere the only synapses on mitral cells are the "reciprocal synapses" with the granule cells (Jackowski*et al*., 1978; Rall*et al*., 1966). Anatomical research has suggested that there are inhibitive synapses on mitral cells (Crespo *et al*., 2001). The mitral cell olfactory neuron synapse is within a tangle of axons and dendrites that is called glomerulus. There is a second cell type tucked around these glomeruli, which probably affects how the signal is transmitted. These cells are

small and densely packed, which gives them the name granule cells. However they bear no relation to the granule cells of the cerebellum or cerebral cortex, they are GABAnergic unlike other cells of the same name (Diana*et al.*, 2000). The layers of human olfactory bulb, especially the mitral cell layer are not as clearly defined as those of other animals (Zilles, 2004). In adult a well-defined mitral cell layer is unclear and an internal plexiform layer is not readily apparent. Mitral cells constitute the main, although not the entire output of the bulb, especially at the end of embryonic and early foetal periods (Smith and Jahr, 2002) Olfactory bulb granule cells form two morphologically distinct spines that are spatially segregated along the dendritic tree: large gemmules in the EPL that are the sites of reciprocal dendrodendritic synapses and spines that are purely postsynaptic along the proximal apical and basal dendrites in the GCL (Price and Powell, 1970a; Shepherd and Greer, 1998).

The tufted cells are smaller and more superficially placed. The larger dendrites of these cells break up into making branches to form a compact round bush terminal (Ranson and Clark, 1959). The tertiary ramifications of olfactory nerve fibres interlace with these dendritic branches to form a circumscribed or spherical olfactory glomerulus. In the glomeruli, the olfactory nerve fibres come into contact with dendritic ramifications of the mitral and tufted cells. It is evident that these dendrites must take up and transmit the olfactory impulses (Ranson and Clark, 1959). The mitral cell axons are thicker and coarse and directed into the lateral olfactory stria, while the finer axons of the tufted cells pass through the anterior commissures to the opposite olfactory bulb. The axons of deeply placed granule cells are relatively short and are directed toward the surface of the bulb.

The main intrinsic granule cell in the vertebrate olfactory bulb lacks an axon. Each cell gives rise to short central dendrites and a single long apical dendrite that expands into

the granule cell layer and enters the mitral cell body layer. The dendrite branches terminate within the outer plexiform layer among the dendrites in the olfactory tract (Neville and Haberly, 2004). In mammalian olfactory bulb, granule cells can process both synaptic input and output due to the presence of large spines (Egger, *et al*., 2005).Olfactory axons synapse on dendrites of mitral/tufted cells within the olfactory bulb glomeruli which are also the sites of synaptic and non-synaptic interactions among dendrites of mitral/tufted cells and juxtaglomerular neurons (Christie and Westbrook, 2006; Kosaka and Kosaka, 2006;Schoppa and Westbrook, 2001; Shepherd *et al.*, 2004; Wachowiak and Shipley, 2006).Among mammals olfactory receptor cells differ a little between the Moles (Graziadei, 1973), the dog (Okano *et al*., 1967) and the Sheep (Kratzing, 1970).

Neurogenesis of the olfactory bulb has been reported in mammals (Gilles, *et al*., 2000). In rodents, the cell of olfactory bulb has reportedly reproduced throughout life in the sub-ventricular zone (Redmond *et al*., 1995; Menezes *et al*., 1998; Dutton and Barlett, 2000).

In man, the olfactory bulb is reported to be a solid structure with its cavity represented by central gray mass of neuroglia. Within the gray matter of the bulb are found three types of neurons; the mitral, tufted and granule cells (Shingo *et al*., 2003). Thus, the adult olfactory bulb should comprise neurons at different maturity levels, given the continual arrival of new cells. Future studies are therefore needed in this area to determine the extent to which the neurons produced in adulthood differ from those produced during development. Inhibition generated by granule cells, the most common GABAnergic cell type in the olfactory bulb, plays a critical role in shaping the output of the olfactory bulb. However, relatively little is known about the synaptic mechanisms

responsible for activating these interneurons in addition to the specialized dendrodendritic synapses located on distal dendrites.

In many brain regions, local circuits form the fundamental building blocks that enable neuronal computation. In the olfactory bulb (OB), locally generated inhibitory synaptic interactions play a central role in shaping mitral and tufted cell responses to sensory stimuli (Hamilton and Kauer, 1989). Although the dendritic arborization of granule cells is relatively small (50–200 μm) (Shepherd and Greer, 1998), a single granule cell may receive synaptic inputs from mitral cells several millimeters away (Shepherd and Greer, 1998), providing a theoretical basis for massive synaptic divergence. *In vivo* recordings, however, suggest that unlike glutamatergic mitral cells, inhibitory granule cells are only weakly activated after sensory stimulation (Cang and Isaacson, 2003). These findings raise the possibility that the pattern of interneuronal activation, and thus lateral inhibition onto principal cells, is not regulated predominately by dendrodendritic synaptic inputs but instead may reflect the temporal coincidence of several types of synaptic inputs to granule cells.

2.3Functions of the Olfactory Bulb

In all vertebrates the main olfactory system detects and processes the vast majority of chemical cues that enter the nasal cavity. The system is a chemical categorizer, first detecting a chemical and then cataloguing it in the cortex for later recall. The role of the main olfactory system in the detection and processing of chemosensory cues that trigger innate, hard-wired physiological or behavioural responses is still somewhat controversial but has recently received considerable attention (Kobayakawa*et al.*, 2007; Restrepo*et al.*, 2004; Shepherd, 2006). The functions of olfactory system were

described by Fletcher (2006) to include: the identification of food, predator versus non harmful species and also it plays a role in mating behaviour.

Kensaku (2005) described the role of olfactory bulb in the daily life of mammals. These roles were in agreement with those listed by Fletcher (2006). But in addition he considered the issue of recognition of their mate, parents, offspring and detecting signals for a variety of social behaviours; including the maintenance of territories. The glomeruli present in the first layer of the olfactory have been found to be the sites of synaptic processing in the olfactory system; it contains at least three types of neurons collectively called juxtaglomerular (JG) neurons. The JG neuron in odour perception is poorly understood (Hayar*et al*., 2004).

Chemical features of odour are encoded by the glomeruli in olfactory bulb and each glomerulus respond to one and only one chemical feature of the odour (Bozza and Mombaerts, 2001). This view of Bozza and Mombaerts (2001) is compatible with that of Freeman.It has been estimated that more than 400,000 different compounds are odorous to the human nose. More surprisingly, not two compounds have been found to have exactly the same odour quality. This suggests that the olfactory system can detect and discriminate more than 400,000 different compounds (Kensaku, 2005). The synapse between mitral and granule cells are dendrodentritic, which means that both sides of the synapse are dendrites that release neurotransmitter. The mitral cells release the excitatory neurotransmitter glutamate and the granule cells release the inhibitory neurotransmitter Gamma-amino-butyric acid (GABA). Glutamate has been reported as the olfactory cell neurotransmitter in turtle, toad and in rat. Noradrenaline has been shown also to be a neurotransmitter in rat olfactory bulb (Berkowicz and Trombley, 2000). Dopamine may play an important neuromodulatory role in olfaction by reducing the transmitter released from olfactory receptors neuron (Berkowicz*et al*., 1994). Both

behavioural and molecular studies pointed out the potential important role dopamine plays in olfaction (Berkowicz*et al.*, 1994)

The olfactory bulb, a part of the olfactory brain, serves as a relay station for primary olfactory neurons located within the nose. Here axons of the primary neurons, mainly the mitral and tufted cells, within the olfactory bulb glomeruli where they closely associate with afferent axon bundles of olfactory receptor neurons (Field *et al.*, 2003). Food acquisition in carnivores is by tracking and catching prey, an activity that demands for well-developed olfactory cue (Walker, 1975). Though plant eating herbivores may require less of the olfactory sense, mediation of reproductive activities such as mating and oestrus and mother-infant interaction are dependent on olfaction (Gelez and fabre-Nys, 2004; Levy, *et al.*, 2004)

Comparative data on the morphometry of the olfactory bulb and its projection structures in animals of diverse feeding and reproductive lifestyles is largely lacking. Furthermore, changes in parameters of certain brain parts have been attributed either to an increase in the number of neurons, the size of such neurons and or their connectivity (Kaas, 2000).

The olfactory bulb provides one of the direct links between the peripherally located olfactory receptors and the primary olfactory cortex and therefore offers connection between the brain and the environment (Turetsky*et al.*, 2000). Given that a larger olfactory bulb volume is indicative of better olfactory function (Haehner*et al.*, 2008), it is possible that the volume of the bulb directly relates with the functional needs and therefore the behavioural ecology of the species. The dog senses are attuned for a wide range of activities including food searching (Tracking and catching prey) and reproduction (Walker, 1975Gittleman, 1989) and therefore require a markedly large olfactory cortex (Coren, 2004) as well as a prominent olfactory bulb. In the goat,

however, the role of olfaction has been narrowed down to cater for reproductive activities including mating and oestrus (Gelez and Fabre-Nys, 2004) and mother-neonate interaction (Levy *et al*., 2004). This explain why the olfactory structures are relative less prominent.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals

Twenty four adult African Giant rats (12 males and 12 females) and twenty four adult Wistar rats (12 males and 12 females), were used for the study. Live African Giant rats were captured directly from the wild in Zaria and its environs. They were transported to the animal house in the Department of Veterinary Anatomy, Ahmadu Bello University (ABU), Zaria using laboratory African giant rat cages, while the Wistar rats were bought from Veterinary Physiology laboratory, A.B.U., Zaria and were kept in a standard laboratory Wistar rat cages. The animals were acclimatized for two weeks before commencement of the experiment during which they had access to food and water *ad libitum.* They were physically examined and only apparently healthy rats were used for this study.

3.2 Extraction and Preparation of the Olfactory Bulbs

3.2.1 Fixation and extraction of the brain

The rats (African Giant rats and Wistar rats) were sedated with gaseous chloroform in a confined glass container. They were weighed and their weights recorded using a weigh balance (P1210 Mettler Instruments AG, Switzerland) with a sensitivity of 0.1 g. Thereafter, the animals were euthanized using the methods ofAdeyemo andOke (1990). The rats head were decapitated at the occipito-atlantal joint while the skin and musculature over the skull were removed as much as possible. A slit was then made through the frontal bone using a hand saw to reveal the meninges. Another cut was made connecting the medial canter to allow further penetration of the fixative. These rats heads were immediately put in containers containing Bouin's solution for 24 hours to facilitate extraction of the brains (Gridley, 1960). The brain extraction was done according to method described by Ramaswamy (1978) using 2 mm jaw face rougeurs knife, spatula and scissors. The OB was secured after cutting the olfactory nerve rootlets passing through the cribriform plate of ethmoid bone. The olfactory bulbs were gentle removed from the ethmoidal fossa. After extraction, the meninges were carefully removed to expose the brain. The whole brain was weighed and recorded (in grams using a Mettler Instrument AG, Switzeland) with a sensitivity of 0.01g and photographs of the two brains were taken.

3.2.2 Separation of the olfactory bulb from the whole brain.

The olfactory bulb was obtained after a cut was made separating the olfactory penduncles on the ventral aspect of the brain. The olfactory bulb of the two rats were then weighed and recorded.

3.3 Anatomical Studies

3.3.1 Gross study

The gross features of the olfactory bulbs such as the shapes, sizes, surfaces and attachments were observed and described.Definitions of gross anatomical structures were based on standard information on rodent anatomy (Parker and Haswell, 1974; Olds and Olds, 1979;Rowett, 1979; Suckow*et al.*, 2006). NominaAnatomicaVeterinria (2005) was used for the nomenclature.

3.3.2 Morphometric study

Brain and olfactory bulb weights were measured with Mettler balance P 1210 (Mettler instrument AG, Switzerland) with a sensitivity of 0.001g. Measurements of lengths, widths and heights were obtained using vernier caliper MG6001 DC (General Tools and Instruments Co., New York) with sensitivity of 0.001cm. The volume of the brain and olfactory bulb were obtained by water displacement method using calibrated cylinder (Scherle, 1970)

3.3.3 Histological study

The olfactory bulbs fixed in Bouin's solution were placed in mould containers (plastic basket like containers).They were washed twice in water for 30 minutes each to reduce the Bouin's solution in the samples. They were now dehydrated in 70%, 80%, 95%, 100% and 100% alcohol each for 24 hours; followed by clearing in xylene for 2 hours. Paraffin wax embedding method as modified was used to block the samples. The tissue blocks were now trimmed and sectioned at 5µm thick in transverse, coronal and longitudinal planes using Jung rotary microtome (Model 42339). Different staining methods were used to view the cells (Tufted, mitral or brush, granular, juxtaglomerular and periglomerular cells), cell layers (Glomerular, granular, mitral/tufted, external plexiform and internal plexiform layers), and cell organization. Stains used were(i)

hematoxylin and eosin (H & E) as routine stain (ii)Cresyl Fast Violet and Enarson's stain for Nissl body staining. Slides were viewed using light microscope at various magnifications. Photomicrographs were taken for comparison using digital camera (SONY®Cybershot, DSC-W110, 7.2 MP, x 4 optical zoom, Japan).

3.4 Data Analysis

Weights, lengths, widths, heights and volume were recorded as mean (Mean ± S.E) and subjected to Student's t-test and Pearson's correlation analysis. Values of $P < 0.05$ were considered significant. Graph pad prism method was used for statistical analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Weights and Dimensions

The value of the mean brain weight of AGR male and female were 3.86±0.01g and 3.33±0.05g,respectively.There was no significant difference ($P > 0.05$) between the brain weight of the male (3.86±0.01g) and female (3.33± 0.05g) AGR (Table 4.2). The brain weight of the male (1.37± 0.01g) and female (1.31± 0.02g) Wistar rats did not also significantly differ between each other (Table 4.4).

The mean brain length of the AGR was 35.39± 0.96mm while that ofWistar rat was 20.54± 0.15 mm (Table 4.1 and 4.4).There was no significant ($P > 0.05$) difference in the brain length of the male (39.69± 0.12 mm) and female (31.09± 0.67 mm) AGR (Table 4.2). The brain length of the male (21.45± 0.15 mm) Wistar rat was slightly higher than that of the female (19.63± 0.26 mm), but the difference was not significant (Table 4.4). The male AGR has a slightly higher brain width, height and volume

compared to the female AGR. The male wistar rat also has its brain width, height and volume slightly greater than those of the female wistar rat, though the differences were not significant (Table 4.2 and 4.4).

The mean left and right Olfactory bulb length, weight, height and volume of the male AGR are slightly higher than those of the female AGR. The left and right olfactory bulb length, weight, height and volume of the male Wistar rat are also slightly higher than those of the female (Table 4.1 and 4.4).

There were significant ($P < 0.05$) correlations between the body weight and the brain weight ($r= 0.5677$), brain length ($r= 0.5803$), brain width ($r= 0.6878$) and brain volume ($r= 0.6400$) of AGR. But there was no significant correlation ($P > 0.05$) between the body weight of the AGR and the brain height ($r= 0.2252$)(Table 4.5). While in the wistar rat, there were significant ($P < 0.05$) correlations between the body weight and the brain length ($r= 0.7749$), brain width ($r= 0.8531$), brain height ($r= 0.8372$) and brain volume ($r= 0.7200$)(Table 4.6).

Significant ($P < 0.05$) correlation was recorded between the brain weight of the AGR and the brain length ($r= 0.8899$) and brain width ($r= 0.8661$). There was however, no significant ($P > 0.05$) correlation between the brain weight of AGR and the brain height ($r= 0.1634$) and brain volume ($r= 0.2388$)(Table 4.5). In the wistar rats however, there were significant ($P < 0.05$) correlation between the brain weight and the brain length ($r= 0.5640$), brain width ($r= 0.6518$), brain height ($r= 0.4758$) and brain volume ($r= 0.4800$)(Table 4.6).

Furthermore, there was a significant ($P < 0.05$) correlation between the brain length of the AGR and the brain width ($r= 0.7039$), however, there were no significant ($P > 0.05$) correlation between the brain length and brain height ($r= 0.2479$) and brain volume r=

0.1698) of the AGR (Table 4.5). But in the wistar rats, there were significant correlation between the brain length and the brain width (r= 0.7243), brain height (r= 0.8184) and brain volume (r= 0.5517) (Table 4.6).

The brain width of the AGR was not significantly correlated with the brain height (r= 0.3281) and brain volume (r=0.2817)(Table 4.5). But in the Wistar rat, the brain width was significantly ($P < 0.05$) correlated with the brain height (r= 0.7485) and brain volume (r= 0.7420)(Table 4.6).

The brain height of the AGR did not significantly correlate with the brain volume (r= 0.1693)(Table 4.5) while the brain height of the wistar rat significantly ($P < 0.05$) correlated with the brain volume (r= 0.6428)(Table 4.6).

The Olfactory bulb width of AGR was not significantly ($P > 0.05$) correlated with the OB height (r= 0.1792)(Table 4.8), the OB width of the wistar rat was also not significantly ($P > 0.05$) correlated to the OB height (r= 0.09544)(Table 4.7). The OB height of the AGR significantly ($P < 0.05$) correlate with the OB volume (r= 0.396)(Table 4.8), the OB height of the wistar rat also did not significantly correlate to the OB volume (r= 0.2248) of wistarrat(Table 4.7). There was no significant correlation between the OB volume of the AGR and the OB weight (r= 0.3066)(Table 4.8), while also in the wistar rat, the OB volume did not significantly correlate with the OB width (r= 0.2921)(Table 4.7).

The left Olfactory bulb weight did not significantly ($P> 0.05$) correlate with the left OB length (r= 0.2133). The right OB weight also did not significantly ($P > 0.05$) correlate with the right OB length (r= 0.1653) in Wistar rat (Table 4.7). There was also no significant correlation in the OB parameters of AGR (Table 4.8).

4.2 Morphological (Gross) Features

4.2.1 The brain

The Olfactory bulb of African giant rat is well protruded and located at the frontal lobe of the telencephalon (Plate I). The OB has a rough pyramidal shape in AGRwhile in wistar rat the OB has crescent–like shape when viewed mid-saggitally(Plate III). The brains of AGR and wistar rat aredevoid of prominent gyri and sulci.In wistar rat, the OB is also located at the frontal lobe of the cerebral hemisphere but small and not well protruded as in AGR (Plate I). Dorsally, the OB, the cerebral lobes, the cerebellum, thevermis, theflocculus and the medulla oblongata were prominent in both rats. Longitudinal and transverse cerebralfissures were conspicuous in AGR and wistar rat brains (Plate I).

Ventrally, the OB of AGR was more prominent than that of wistar rat. Through surface of the OB of AGR was due to torn olfactory nerves during the process of removal of the

brain (Plate II). This perhaps signifies that the OB of AGR is well innervated with olfactory nerve from the nasal epithelium than that of the Albino rat. The large size of the OB of AGR signifies the macromastic nature of AGR. In both rats, the OB gave branches into medial, lateral and middle olfactory tracts that conveyed sensory impulses to olfactory cortex.The mid-sagittal view also showed the larger size of OB of AGR. (Plate III).

TABLE 4.1: Mean values of brain and OB parameters of Male and Female AGR (n =24)

	Brian	OB	Percentage (%) of OB/Brian
Weight	3.60±0.06	1.25±0.06	54.72
Length	35.39±0.96	5.97±0.04	16.84
Width	18.44±0.38	2.82±0.06	2.06
Height	12.54±0.06	4.31±0.09	34.30
Volume	6.03±0.07	0.22±0.005	3.64

Key: OB = Olfactory bulb

Table 4.2: Mean values of brain and OB parameters of male and female AGR

Mean values of brain and OB parameters of male AGR (n = 12)				Mean values of brain and OB parameters of female AGR (n = 12)			
	Brain	**OB**	**Percentage (%)OB/Brian**		**Brain**	**OB**	**Percentage (%)OB/Brain**
Weight	3.86±0.01	1.27±0.01	32.90	**Weight**	3.33±0.05	1.26±0.01	37.83
Length	39.69±0.12	6.01±0.02	15.14	**Length**	31.09±0.67	5.92±0.07	19.04
Width	20.17±0.24	2.82±0.10	13.98	**Width**	16.72±0.13	2.81±0.06	16.81
Height	12.62±0.09	4.61±0.14	36.52	**Height**	12.46±0.08	4.01±0.04	32.18
Volume	6.05±0.10	0.22±0.007	3.63	**Volume**	6.03±0.02	0.22±0.02	3.65

Table 4.3:Mean values of brain and OB parameters of female and male wistar rats (n =24)

	Brian	OB	Percentage (%) of OB/Brian
Weight	1.34±0.01	0.04±0.001	2.98
Length	20.54±0.24	3.97±0.06	17.47
Width	12.19±0.13	1.55±0.01	12.71
Height	7.48±0.15	3.21±0.05	42.91
Volume	2.20±0.004	0.12±0.002	5.45

TABLE 4.4:Mean values of brain and OB parameters of Female and Male WR

Mean values of brain and OB parameters of Female WR (n = 12)				Mean values of brain and OB parameters of Male WR (n = 12)			
	Brain	**OB**	**Percentage OB/Brian**		**Brain**	**OB**	**Percentage OB/Brain**
Weight	1.31±0.02	0.04±0.07	3.05	**Weight**	1.37±0.01	0.04±0.001	2.92
Length	19.63±0.26	3.40±0.09	17.32	**Length**	21.45±0.15	3.79±0.02	17.67
Width	11.71±0.12	1.51±0.02	12.89	**Width**	12.68±0.12	1.58±0.01	12.46
Height	6.79±0.09	3.28±0.04	48.31	**Height**	8.17±0.08	3.13±0.08	38.31
Volume	2.02±0.02	0.11±0.002	5.31	**Volume**	2.33±0.06	0.12±0.003	5.15

Table 4.5: Correlation between different brain parameters in the African Giant Rats

African Giant rat Correlation					
Brain parameters	**Brain weight**	**Brain length**	**Brain width**	**Brain Height**	**Brain Volume**
Body weight	r = 0.5677 p = 0.0038	r = 0.5803 p = 0.0030	r = 0.6878 p = 0.0002	r = 0.2252 p = 0.2901	r = 0.6400 p = 0.0008
Brain weight	1	r = 0.8899 p < 0.0001	r = 0.8661 p < 0.0001	r = 0.1634 p = 0.4454	r = 0.2388 p = 0.2612
Brain length		1	r = 0.7039 p < 0.0001	r = 0.2479 p = 0.2426	r = 0.1698 p = 0.4277
Brain width			1	r = 0.3281 p = 0.1176	r = 0.2817 p = 0.1823
Brain height				1	r = 0.1693 p = 0.4291
Brain volume					1

Table 4.6: Correlation between different brain parameters in the Wistar Rats

Wistar rat Correlation					
Brain parameters	**Brain weight**	**Brain length**	**Brain Width**	**Brain Height**	**Brain Volume**
Body weight	r = 0.6991 p < 0.001	r = 0.7749 p < 0.0001	r = 0.8531 p < 0.0001	r = 0.8372 p < 0.0001	r = 0.7200 p < 0.0001
Brain weight	1	r = 0.5640 p = 0.0041	r = 0.6518 p = 0.0006	r = 0.4758 p = 0.0188	r = 0.4800 p = 0.0176
Brain length		1	r = 0.7243 p < 0.0001	r = 0.8184 p < 0.0001	r = 0.5517 p = 0.0052
Brain width			1	r = 0.7485 p < 0.0001	r = 0.7420 p < 0.0001
Brain height				1	r = 0.6428 p = 0.0007
Brain volume					1

Table 4.7: Correlation between olfactory bulb parameters in Wistar Rats

Wistar rat Correlation							
OB parameters	**OB width**	**OB Height**	**OB volume**	**Left OB weight**	**Right OB weight**	**Left OB length**	**Right OB length**
OB Width	1	r = 0.09544 p = 0.6573					
OB Height		1	r = 0.2248 p = 0.2909				
OB Volume			1	r = 0.2921 p = 0.1660			
Left OB weight				1	r = 0. p = 0.		
Right OB weight					1	r = 0.2133 p = 0.3171	
Left OB length						1	r = 0.1653 p = 0.4401
Right OB length							1

Keys: OB = Olfactory bulb

Table 4.8: Correlation between olfactory bulb parameters in African Giant Rats

African Giant rat Correlation							
OB parameters	**OB width**	**OB Height**	**OB Volume**	**Left OB weight**	**Right OB weight**	**Left OB length**	**Right OB length**
OB Width	1	r = 0.1792 p = 0.4021					
OB Height		1	r = 0.396 p = 0.0553				
OB Volume			1	r = 0.3066 p = 0.1451			
Left OB weight				1		r= 0.1245 p= 0.2305	
Right OB weight					1	-	r= 0.2034 p= 0.1062
Left OB length				r= 0.1872 p= 0.4502	-	1	-
Right OB length				-	r= 0.2010 p = 0.3650	-	1

Keys: OB = Olfactory bulb

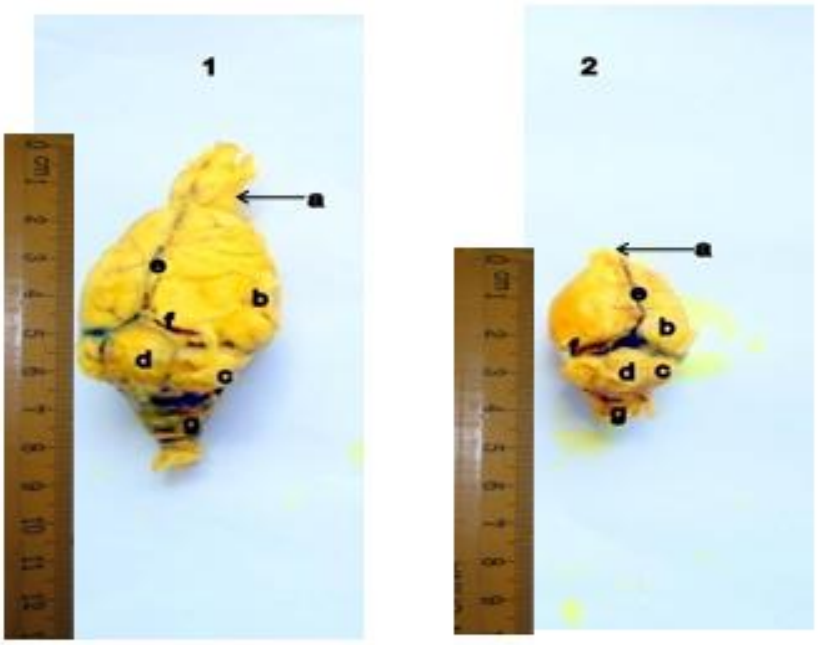


Plate I: Dorsal views of the brain of African Giant Rat (1) and Wistar rat (2), showing; olfactory bulb (a), cerebrum(b), flocculus (c) , vermis(d),longitudinal fissure(e), transverse fissure(f), medulla oblongata(g).

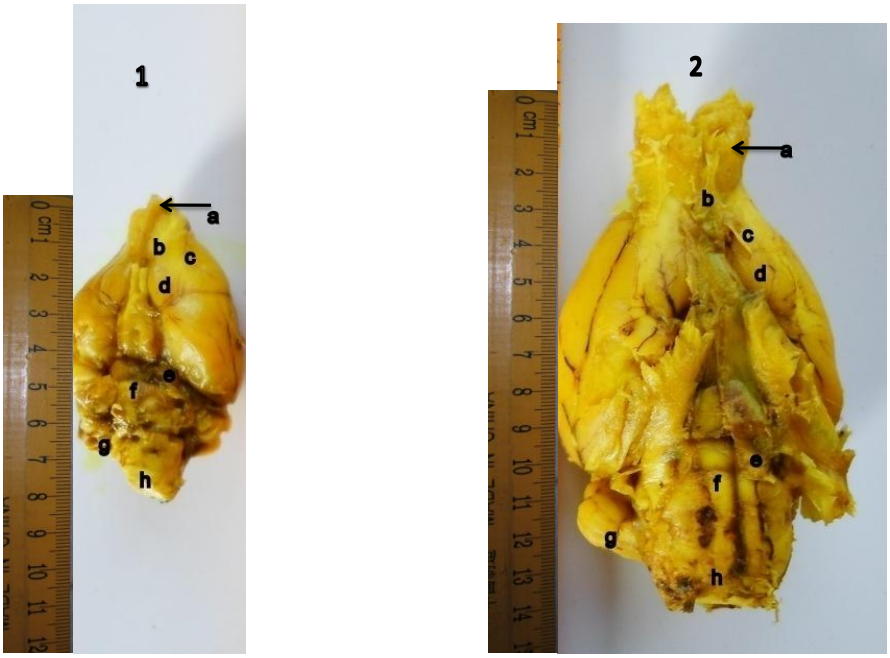


Plate II: Ventral views of the brain of Wistar Rat (1) and African Giant Rat (2), showing Olfactory bulb(a), Medial olfactory tract (b), Lateral olfactory tract (c), Middle olfactory tract (d), Cerebral crus (e), Trapezoid body (f), Paraflocculus (g), Medulla Oblongata (h)

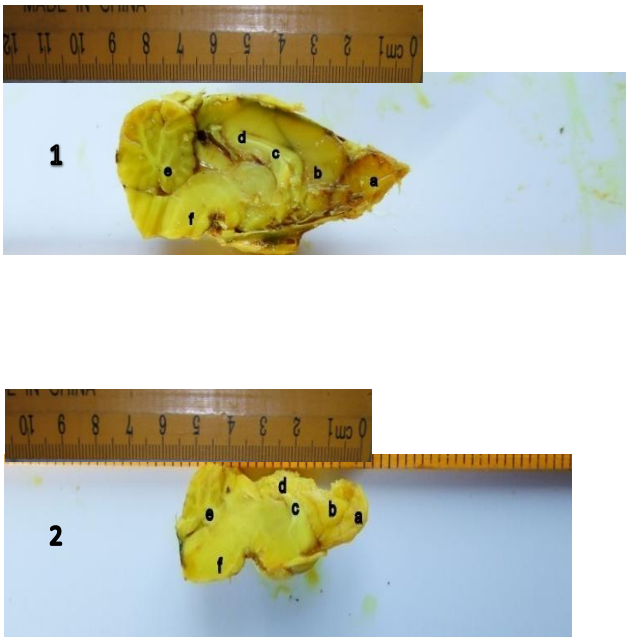


Plate III: Mid-sagittal views of the brains of African Giant Rat (1) and Wistar Rat (2); olfactory bulb (a), corpus collosum (genu) (b), interthalamic adhesion (c), corpus collosum (d), arbor vitae (e) and medulla oblongata (f)

4.2.2 Histomorphological findings

The Olfactory Bulb of African Giant Rat (AGR)and Wistar Rat (WR) have the same concentric laminar organisation showing 6 layers as found in other mammals. These layers in both rats can easily and clearly be distinguished. The layers are,Olfactory Nerve Layer, Glomerular Layer, External Plexiform Layer, Mitral Cell layer, InternalPlexiform Layer and Granular Cell layer. Both Rats have a central region called the Medulla

The Cresyl fast violet stain was able to bring out the neuronal structure of OB hence the laminar organisation as shown in Plates IV & V in both rats. H and E stain also gave a good laminar organisation of the various layers of the OB as shown in Plates VI and VII

When you look atPlates IV, V, VI, and VIIthe 2 stains brought out clearly the 6 concentric laminar layers of the OB of AGR and WR.

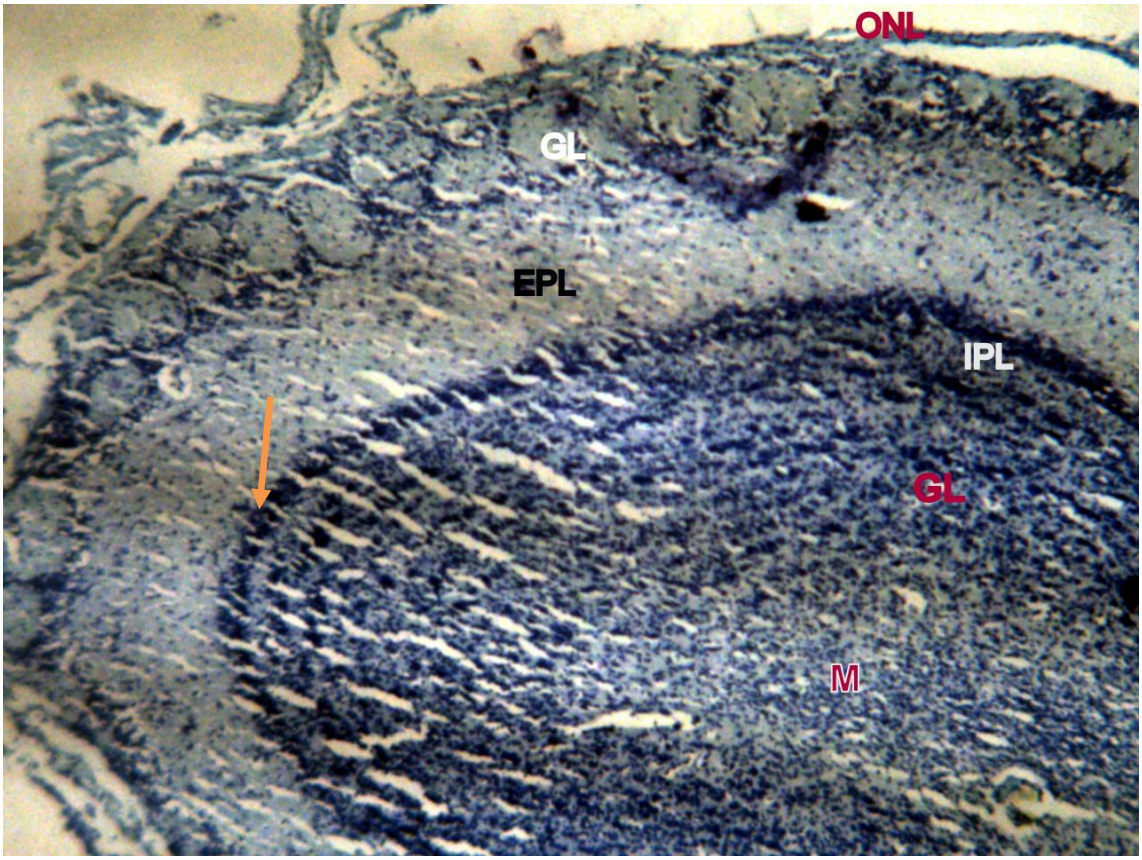


Plate IV: Transverse section of OB inAGR showing the various layers; Olfactory Nerve Layer (ONL), Glomerular Layer(GL), External Plexiform Layer(EPL), Mitral Cell Layer (arrow), Internal Plexiform Layer (IPL), Granule Cell Layer (GCL)and Medulla(M). Cresyl fast violet stain X 40.

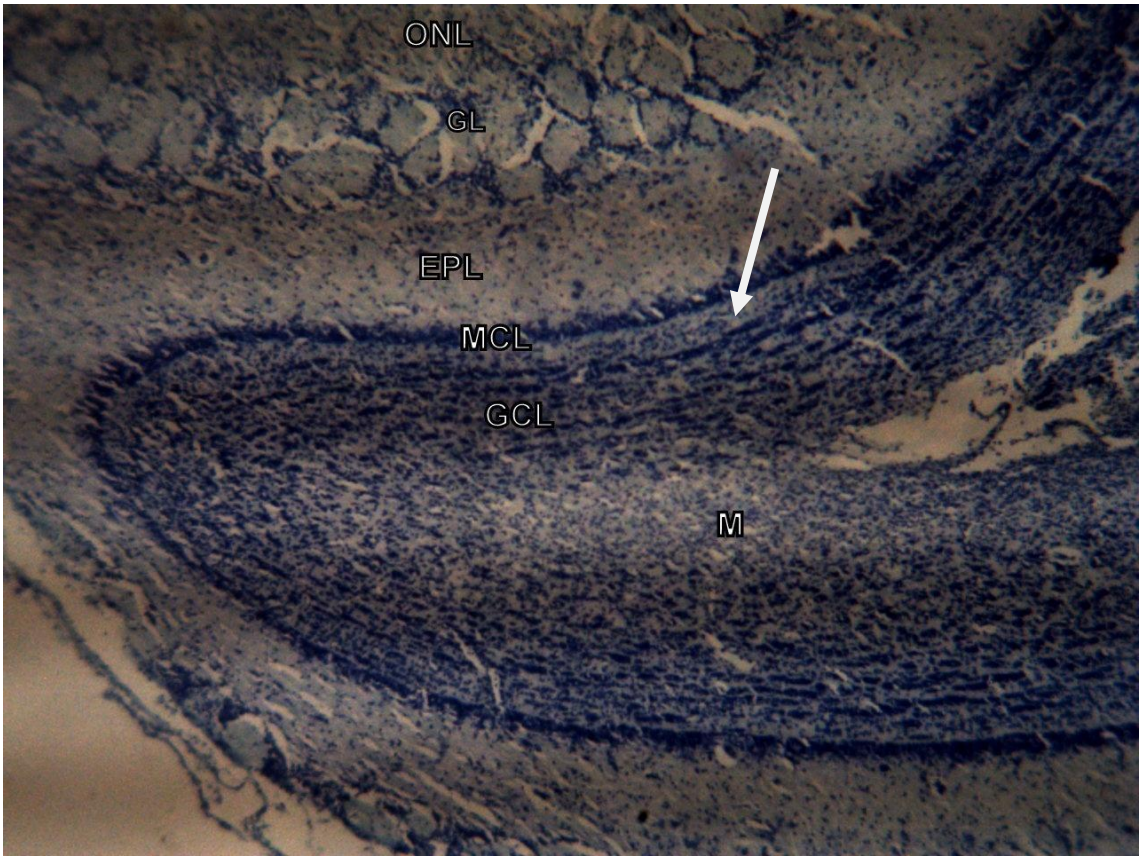


Plate V: Transverse section of OB in WR, showing the various layers namely;Olfactory Nerve Layer (ONL); Glomerular Layer (GL); External Plexiform Layer (EPL); Mitral Cell Layer (MCL); White arrow shows Internal Plexiform Layer (IPL); Granule Cell Layer (GCL) and Medulla (M). Cresyl fast violet stain X 40.

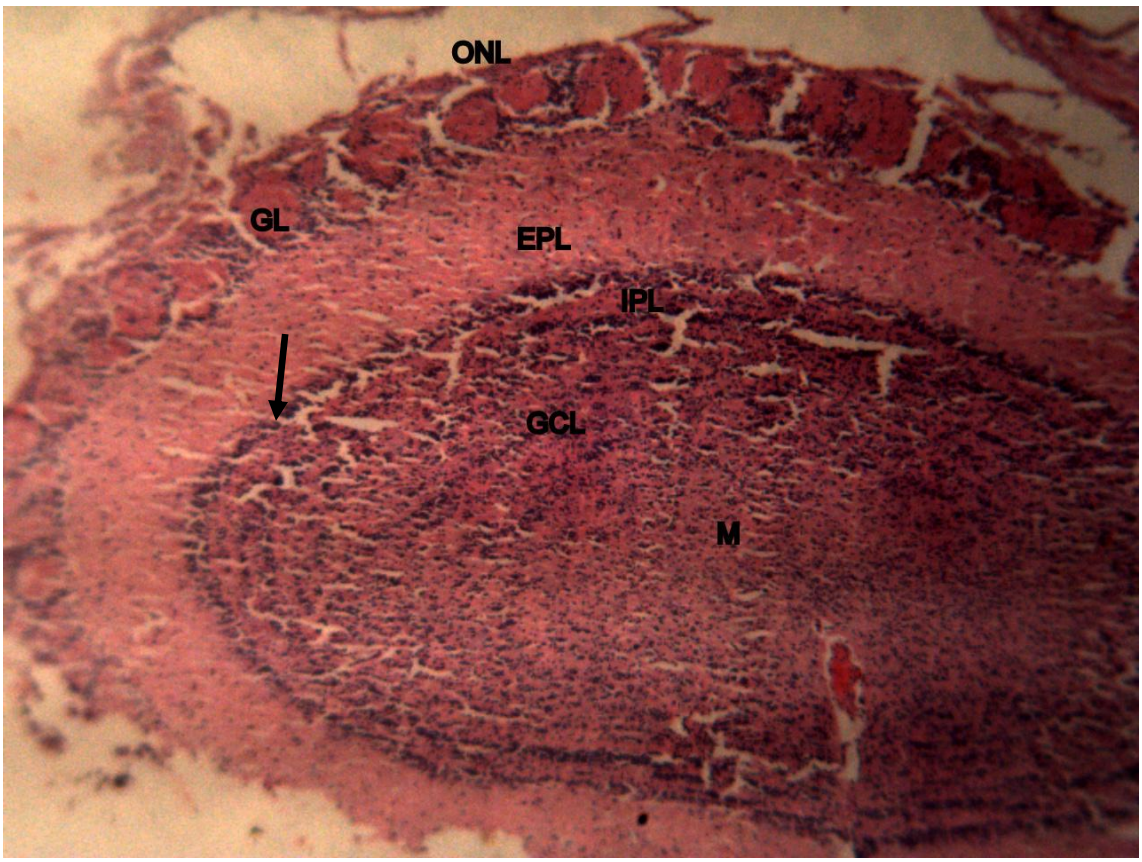


Plate VI: Transverse section of OB in AGR showing the various layers;Olfactory nerve layer (ONL), Glomerular layer (GL), External Plexiform Layer (IPL), Mitral cell layer (Arrow), Internal plexiform layer (IPL), Granular cell layer (GCL), Medulla (M). H and E stain X 40.

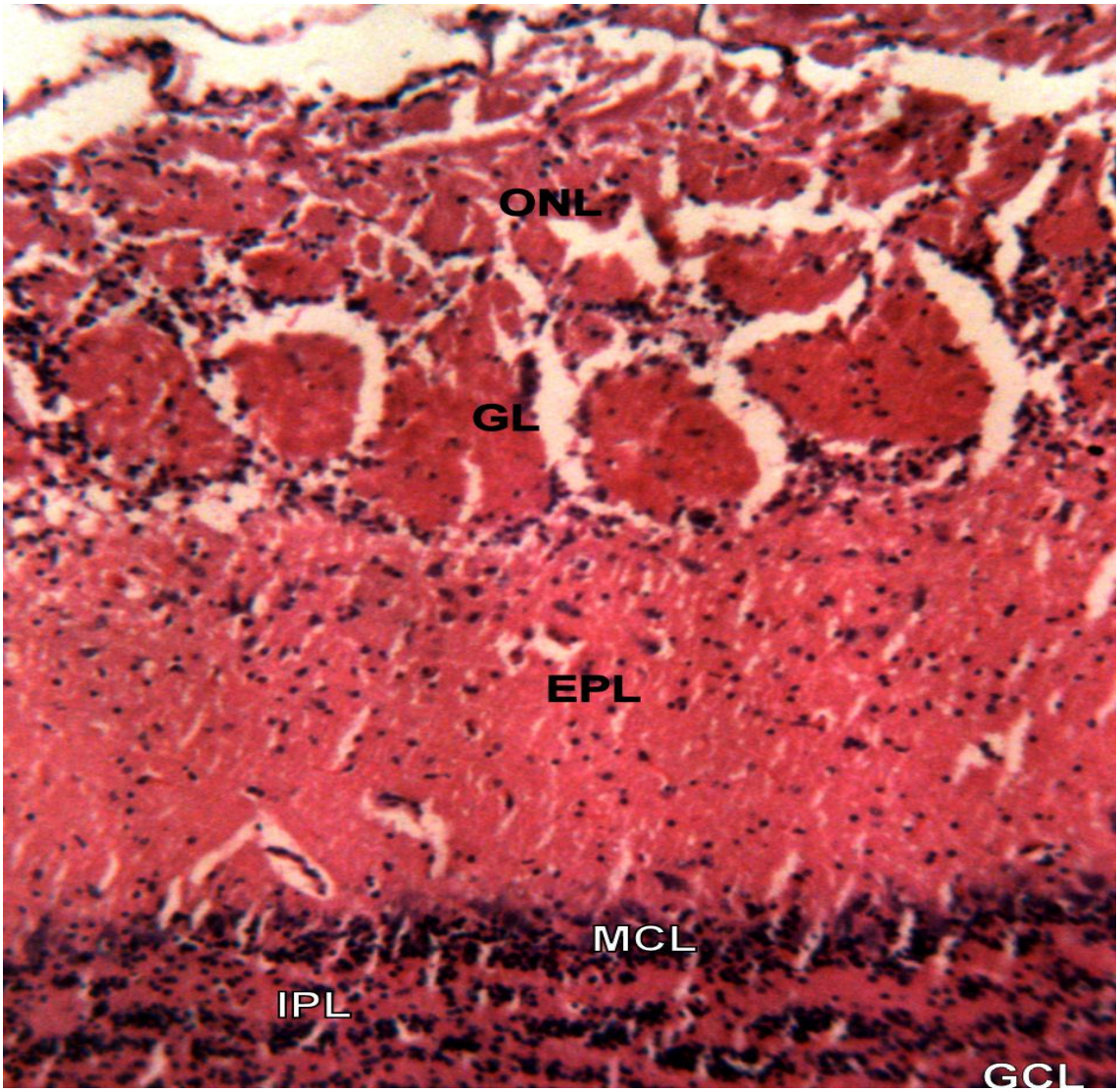


Plate VII: Transverse section of OB inWistar rat showing the various layers;Olfactory nerve layer (ONL), Glomerular layer (GL), External Plexiform Layer (EPL), Mitral cell layer (MCL), Internal plexiform layer (IPL), Granular cell layer (GCL), H and E stain X 250.

4.2.3 Olfactory nerve layer

The Olfactory Nerve Layeris the outermost layer of the olfactory bulb of both Rats. This layer mainly consists of axons that are arranged in groups. Olfactory neurons via the nasal epithelial cells make contact with odour in the atmosphere, and conduct this signal to olfactory glomerular by it axons. Many bundles of fibers formed by olfactory neurons' axon reached the glomerular. (Plates VIII and IX).

4.2.4 Glomerular layer

This layer consists of glomerulars which are made up of synaptic contacts between mitral cell dendrites and olfactory nerve, also with periglomerular cells. In AGR oval olfactory glomerulars were seen in 2 layers thick while in WR the glomerular layer was 3 layers thick. The glomerulars are surrounded by glial cells and periglomerular cells which are interspace neurons and are connected closely with the glomerulars. The olfactory information can be converged at this layer. The olfactory neurons' impulse was conducted to mitral cells, tufted cells and periglomerulars in this layer (Plates IX, X and XI).

4.2.5 External plexiform layer

This layer is made up of primary dendrites of mitral cells and tufted cells, tufted cells and nerve fibres in both rats. This layer is divided into 2; the external and internal sub layers (Plates X and XI).

4.2.6 Mitral cell layer

The narrow Mitral Cell Layer contained Miter-resembling (pyramidal) cell bodies of mitral cells, their axons and dendrites, tufted cells and small quantity of granule cells. Mitral cells are the largest cell in the olfactory bulb and according to literature hasan exciting effect on granule cells while granule cells inhibit mitral cells. The mitral cells were well distributed in the AGR and WR (Plate XII, XIII, XIV and XV).

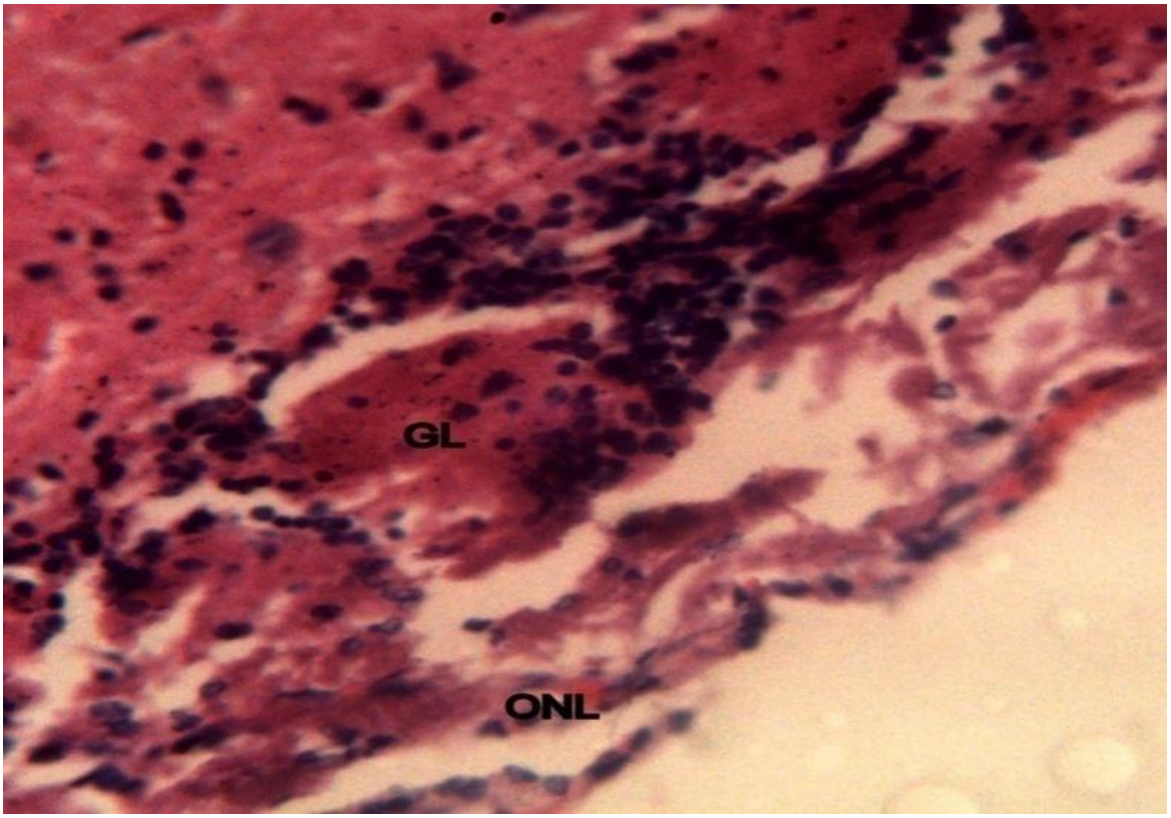


Plate VIII: Transverse section of Olfactory Nerve Layer (ONL)andGlomerular Layer(GL) in AGR. H&E stain X 250.

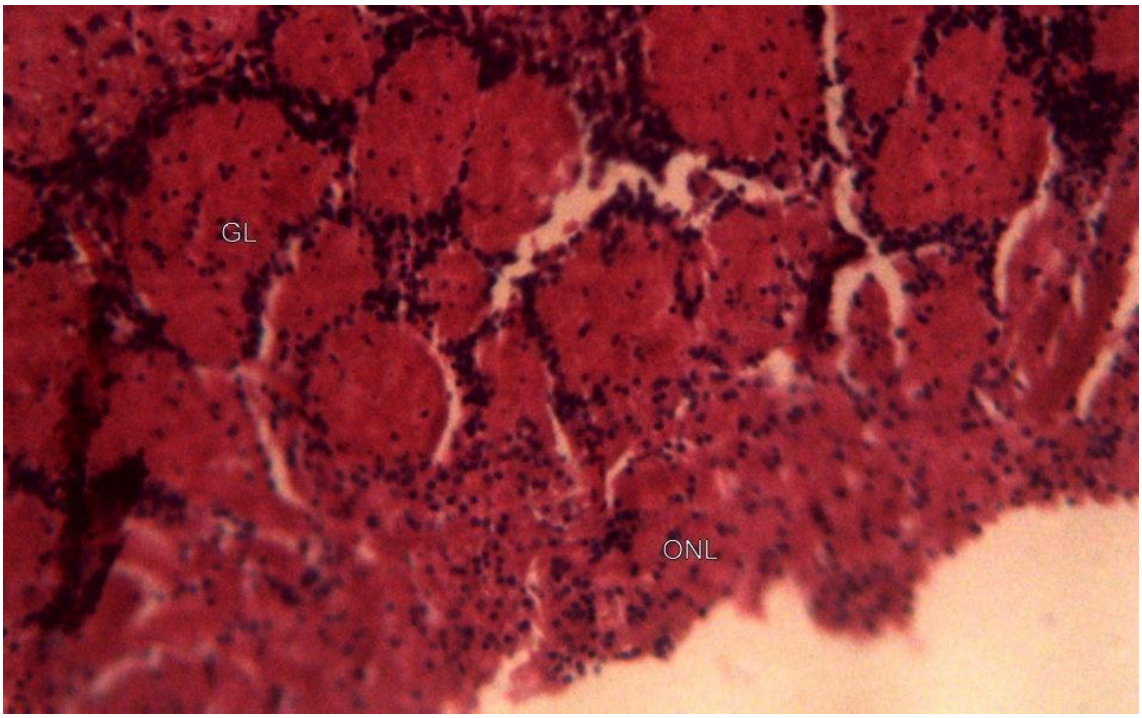


Plate IX: Transverse sectionofOlfactory Nerve Layer (ONL) andGlomerular Layer (GL)in WR. H and E stain X 250.

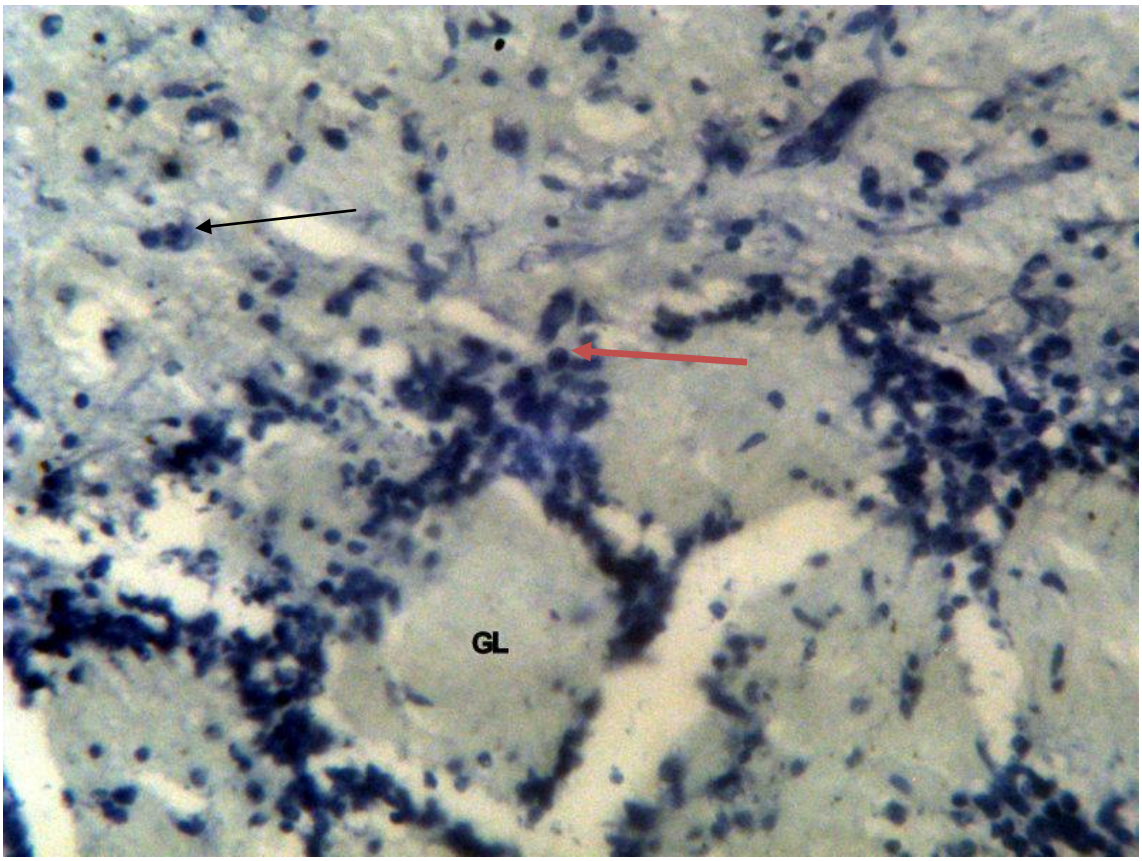


Plate X: Transverse section of theglomerular layer (GL) showingperiglomerular cells (brownarrow), tufted cells (black arrow) glial cells and External Plexiform Layer(EPL) in WR,Cresyl fast violet stain X 250.

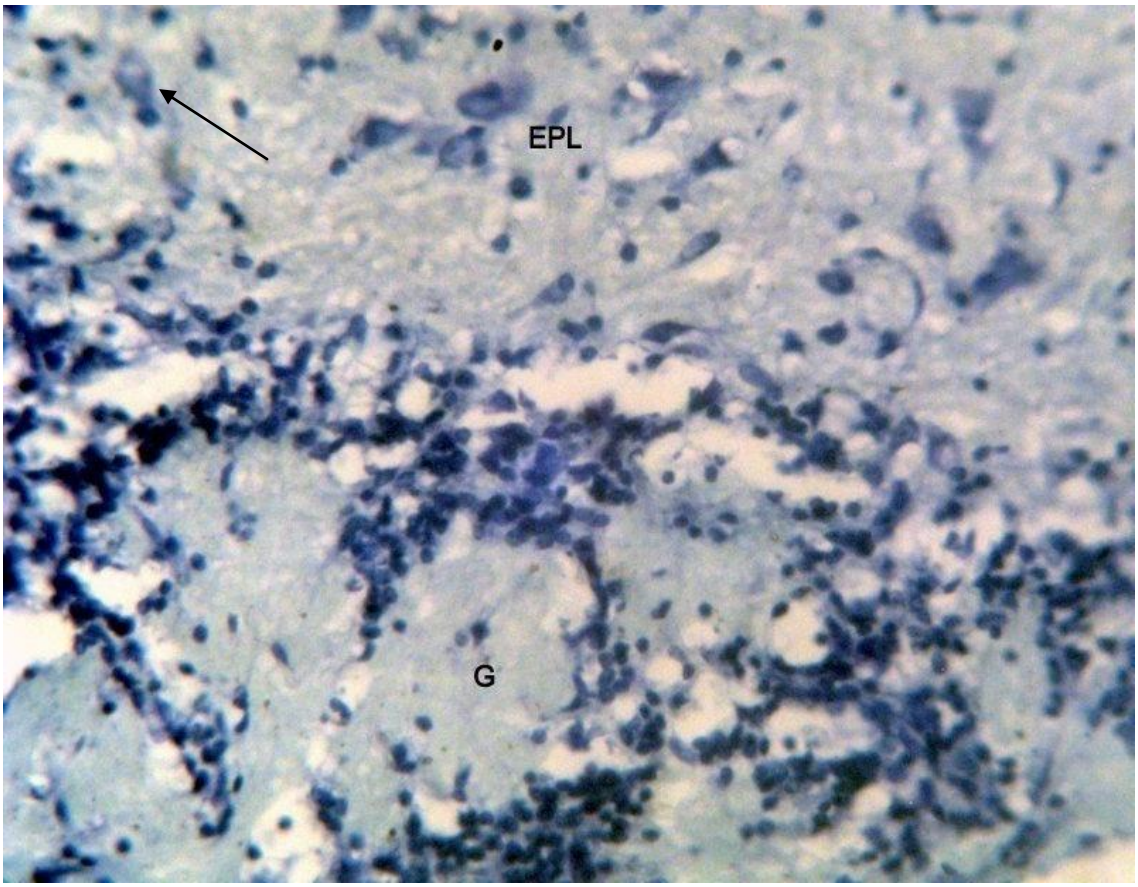


Plate XI: Transverse section of Glomerular (G) surrounded by periglomerular cells, tufted cell (black arrow)and glial cells and ExternalPlexiform Layer (EPL)of AGRCresyl fast violet stain X 250

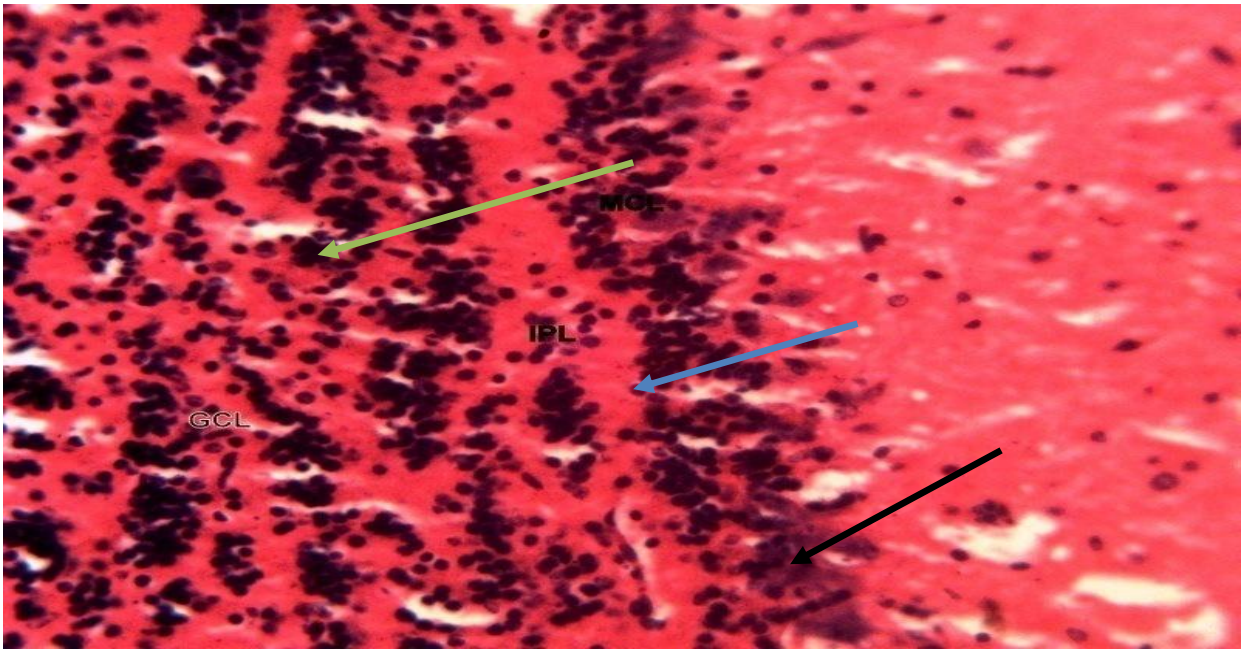


Plate XII: Transverse section of OB in AGR showing Cluster of Mitral cells (black arrow); Tufted cells (blue arrow), Granule cell (green arrow).Internal Plexiform Layer (IPL); Granular Cell Layer (GCL); Mitral Cell Layer (MCL) .H and E X 250.

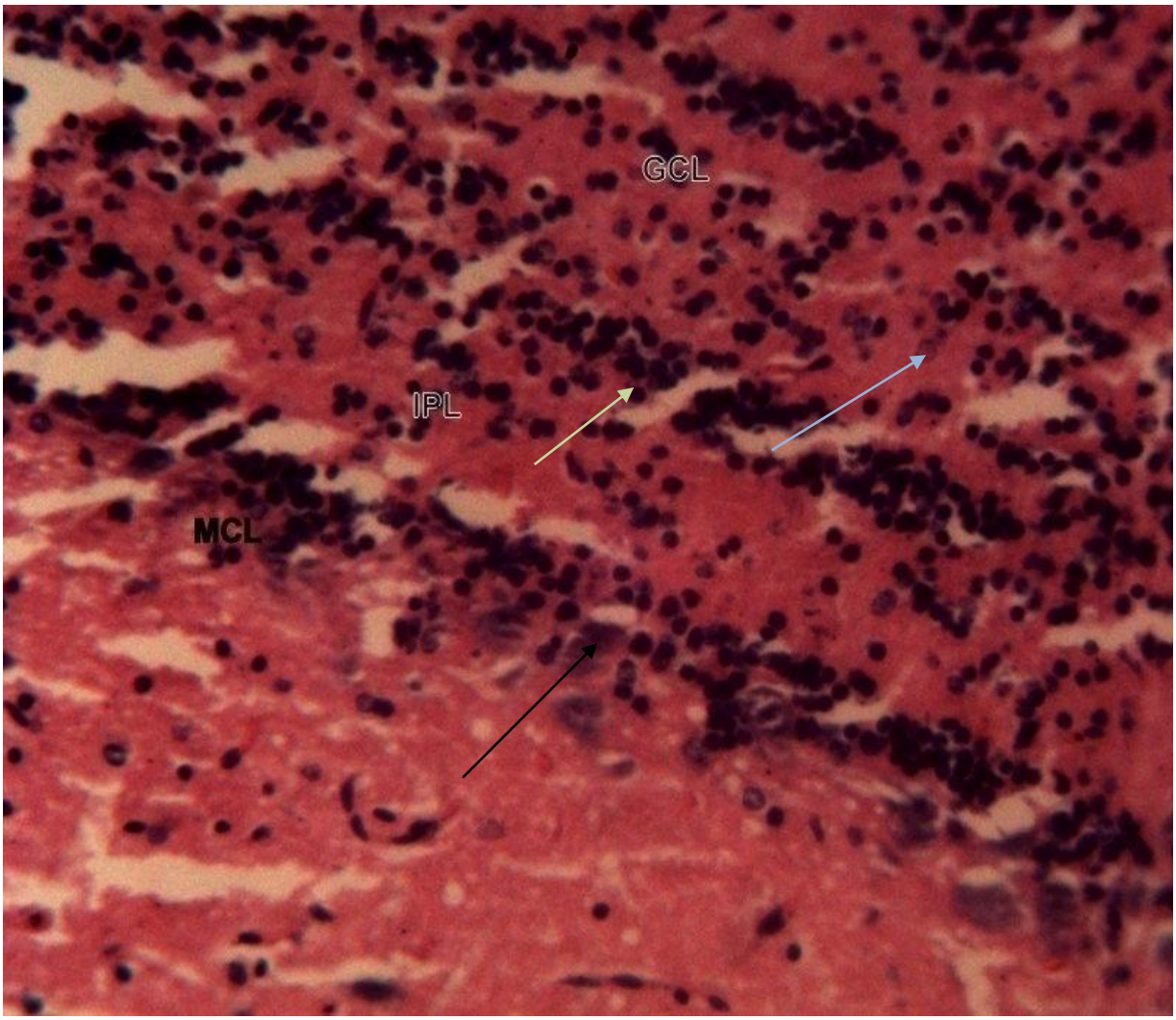


Plate XIII: Transverse section of OB in WR showing Mitral cell (black arrow) with centrally located nucleus; tufted cell (blue arrow) and granule cell (green arrow); Internal Plexiform Layer (IPL); Mitral Cell Layer(MCL) and granular cell layerin WR. H and E X 250.

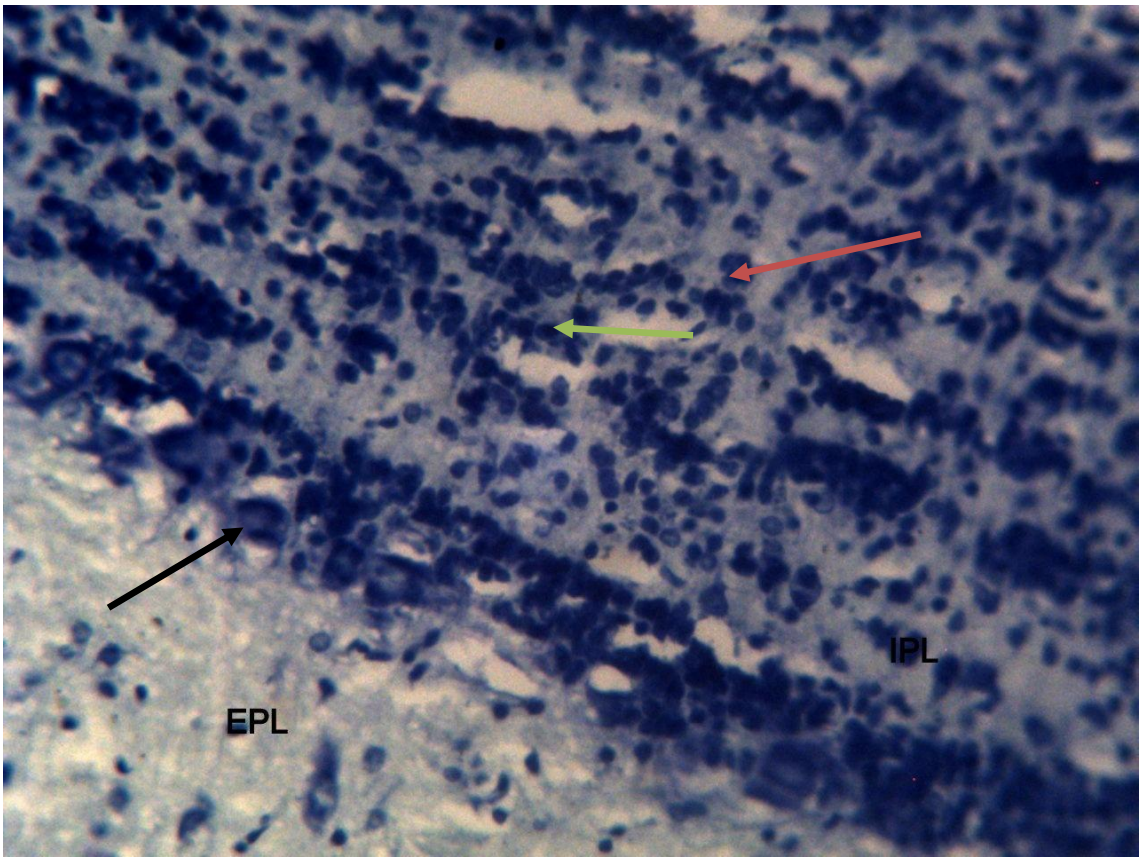


Plate XIV: Transverse section of OB in AGR showing mitral cell (Black arrow), granule (Green arrow) and Tufted cell (Red arrow). Cresyl fast violetstainX 100.

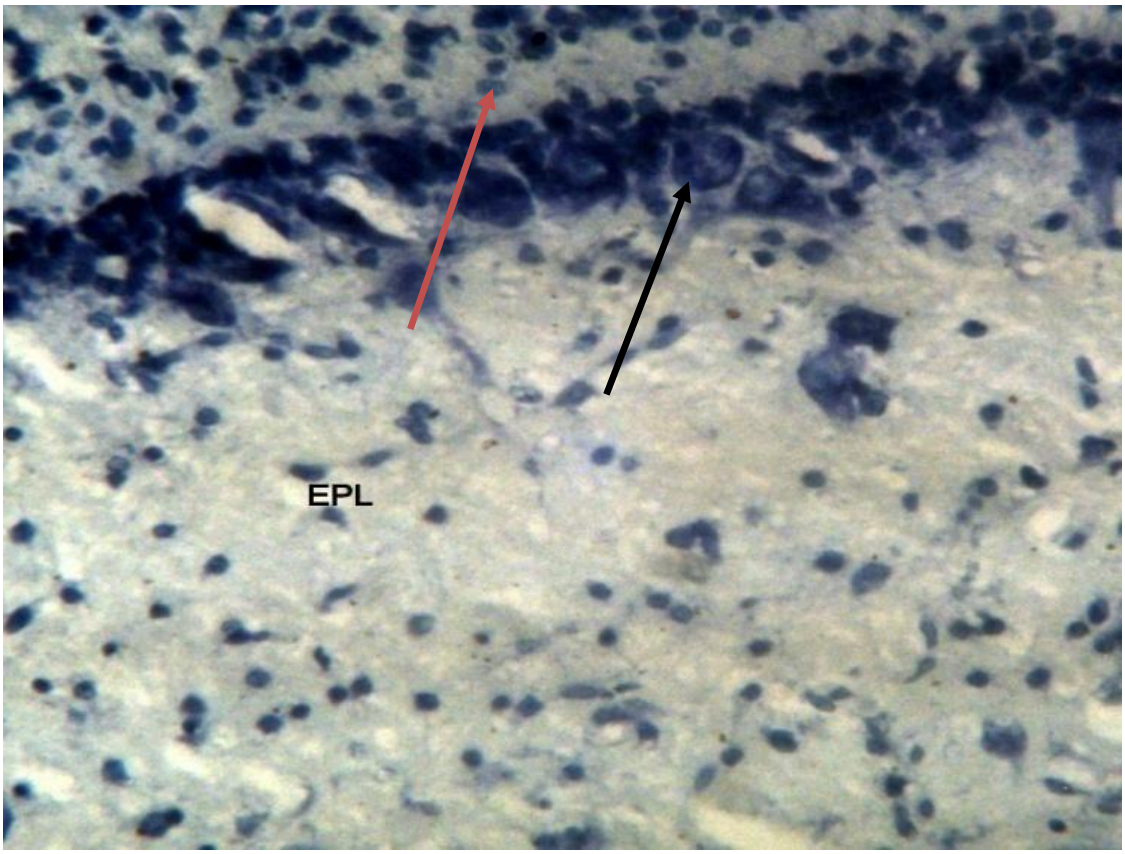


Plate XV: Transverse section of OB in WR showing mitral cell (Black arrow); Tufted cell (Red arrow).Cresyl fast violet X 100.

4.2.7 Internal plexiform layer

This layer is found in both Rats. Tufted and granule cells are the neuronal somata found in this layer. Also found are axons and dendrites of mitral and granule cells.The Internal Plexiform Layer(IPL) like the External Plexiform Layer(EPL) has a low cellular density and comprised scattered tufted cells, granules and dendrites of mitral cells (Plate XII, XIII, XIV and XV).

4.2.8 Granule cell layer

The granule cell layer is the largest layer with most numerous cells of the OB. They are made up of mainly granule cells, their axons and dendrites. Round granule cells are the interspace neurons, and they are in synaptic contact with mitral cells, tufted cells and short axon cells, axons and branches. Of all the neurons of olfactory bulb, the granule cells are morphologically atypical in not having a typical axon but participate in reciprocal synapses with mitral cells (Plate XII, XIII and XIV).

CHAPTER FIVE

5.0DISCUSSION

The essence of the current study on the comparative evaluation of olfactory bulbs in African giant pouched rats (*Cricetomysgambianus*waterhouse) and Wistar rats using different staining techniques was to investigate any possible anatomical differences between the two rats, extrapolate a base-line data so as to document a detailed description of neuroanatomical characteristics with emphasis on morphology, morphometric and histomorphology.

The result in weights and dimension of this study revealed that the mean body weight and brain weight values of the male AGR were greater than those of the females. This is in agreement with the findings of (Ibe*et al*., 2010). Which showed that the value of male AGR brains were higher than that of female AGR. This is also applicable to Wistar rat values. For instance in humans,males' heads are about 2% bigger than female's; this is due to larger physical stature of males. Male's larger muscle mass and larger body size requires more neurons to control them. The brain weight is related to the body weight partly because it increases with increasing height (Ho*et al.*, 1980).

The size of the olfactory bulb of African giant rat was larger than that ofWistar rat (Plate I) this variation is attributed to the degree of olfactory dependency thus agreed with the findings of (Bang and Cobb, 1968). The olfactory bulb provides one of the direct links between the peripherally located olfactory receptors and the primary olfactory cortex and therefore offers connection between the brain and the environment (Turetsky*et al*., 2000). According to (Kavoi*et al*., 2010), the structure of the primary olfactory cells is structurally designed in a way that it meets the olfactory functional

challenges. It therefore seems probable that the size of the olfactory bulb also reflects on the level of dependency on olfaction for survival in a given species.

The prominence and location of the olfactory bulb of both African giant rat and Wistar rat presented proximal to the cerebral hemisphere agrees with observation of (Muller andO'Rahilly, 2004) in rodents and monkey. The bulbs were separated and paired in both African giant rat and Wistar rat thisagrees with observation by (Crosby and Humphrey, 1939). The increased length of olfactory bulb results from the characteristics lengthening and narrowing hemispheres at the level of anterior poles with the observation of (Sisson and Grossman, 1953).

The histological features of the OB in African giant and Wistarrats present thesame 6 concentric lamina layers for the 3 stains. This agrees with the findings of (Nzalak*et al*., 2005;Olude*et al*., 2014), 5 layers were reported in Grasscutter (Byanet*et al*., 2008). Other literature described up to 8 layers Elephant (Marschner, 1970) to include the ependymal layer and an external granule cell layer, located between the glomerular and external plexiform layer in certain species.

The major difference in the olfactory bulb of African giant rat and wistar rat is at the glomerular layer. In African giant rat the glomerular layer was 2 layers thick, this agrees with the findings of (Olude*et al* 2014) while in Wistar rat it was 3 layers thick. This may likely suggest the better olfaction in African giant rat because impulse can easily be conducted faster through 2 layered thick surfaces than a layer that is 3 layers thick. The 6 concentric laminar layers found in both rats disagree with the reports of (Nzalak*et al*., 2005) and (Byanet*et al.,* 2008*)* who ascribed the better olfactory capability of African

giant rat over Grasscutter to the number of layers found in their olfactory bulbs. Literature ascribed olfactory capability to the number of glomeruli and axons of olfactory neurons, mitral and tufted cell that synapse with the glomerulus (Buck and Axel, 1991).Glomeruli are characteristic anatomical features of the first synaptic level of the olfactory pathway in a phylogenetically broad range of animals, including mollusc, arthropods and vertebrates (Ache, 1991). Glomeruli are widely believed to represent functional units in processing olfactory information (Shipley and Ennis, 1996).

In rodents, for example rabbits, there are about 2000 glomeruli receiving inputs from 50 x 10^6 olfactory neurons and each glomerulus is innervated by about 25 mitral cells and 50 tufted cells (principal neurons) (Buck and Axel, 1991; Buck, 1996; Shepherd and Greer, 1998). In mammals, glomeruli typically range between 1100 and 2400 depending on the species, with roughly between 1100 and 1200 in humans (Kosaka*et al*., 1998) while in dog there are about 5000 glomeruli.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Sequel to the findings of this study the following conclusions were made:

A comparative base line data between the two rats were extrapolated giving a detailed description of neuroanatomical characteristics with emphasis on morphology, morphometric and histomorphology.The result of weights and dimensions of this study revealed that the mean body weight and brain parameters of the male African giant rat were greater than those of the females. The male Wistar rat also had its body weight and parameters slightly greater than those of the female Wistar rat.

The mean left and right OBL weight, height and volume of the male AGR and WR were slightly higher than those of the female AGR and WR. The left and right OBL weight, height and volume of the male Wistar rat are slightly also higher than those of the female. The 3 stains gave a clear histology of the concentric laminar organization of the olfactory bulb, showing 6 layers in both rats. Glomerular cell layer in AGR was 2 layers thick, while that of WR was 3 layers thick. In both rats mitral cells were well distributed in the mitral cell layer.

The number of concentric laminar layer in the olfactory bulb is not the reasons for better olfaction but rather the number of glomeruli and axons that synapse with it. Among the 3 stains, cresyl fast violet gave a better histograph of the olfactory bulb layers and cell types.

6.2 Recommendations

Ultrastructural and Immunohistochemical studies should be carried out on the various cells in the olfactory bulb of both rats. This will help in understanding the roles these cells play during olfaction.

Further studies should be carried out to determine and evaluate the macromastic features of the cell organization, cell layers and cell types of the olfactory bulb of African giant rat and other rodents using neurone tracing method and micrometry.

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