NEUROCELLULAR PROFILE IN THE BRAIN OF THE AFRICAN GIANT RAT (*Cricetomys gambianus*, Waterhouse, 1840)

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ABSTRACT

The African Giant Rat (AGR) recently gained international prominence due to its ability to detect landmines and tuberculosis using olfactory and cognitive cues. However, a striking gap exists in the literature on the neurocellular components that govern these unique abilities. This study investigated and described the morphology of cellular components, useful morphometric parameters and neurogenesis profile of the brain of the AGR across age groups.

Forty male AGR comprising 10 neonates, 15 juveniles and 15 adults obtained from the wild were used for this study. Brains were harvested, weighed and studied grossly. Cyto-architectural study on different brain regions was performed using Nissl and Haematoxylin-Eosin stains. Differential staining of sections was achieved using Golgi silver impregnation and Kluver Barrera methods for cortical neurons and myelin morphology respectively. Immunohistochemical identification of individual neurocellular populations was done using the following biomarkers: glia fibrillary acidic protein for astrocytes, 2', 3' cyclic nucleotide phosphodiesterase for oligodendrocytes, ionizing calcium binding adhesion molecule-1 for microglia, doublecortin for immature and migrating neurons and Ki-67 for proliferative neuronal cells. Histomorphometric analysis was carried out using the Axioskop and TSview photomicrographs. Findings were compared across age groups and quantitative data were analysed using One-way ANOVA at $p = 0.01$.

Gross morphology showed part insertion of the falx cerebri between the olfactory bulbs (OB). Cyto-architecture revealed a persistent external granular layer in the post-neonatal cerebellum and multilayered ependymocytes in the ventricular lining. The presence of 4 to 8 glomerular cell layers and myelination across all layers were observed in the OB. Dense spiny multipolar populations of cortical neurons were found in layers II, III and V of the cerebral cortex. Astrocytes demonstrated age related changes and morphological heterogeneity with eight sub-types. Five oligodendrocyte and two microglia classes were identified. Doublecortin and Ki-67 positive cells were demonstrated in the active neurogenic sites (sub-ventricular zone (SVZ) and dentate gyrus (DG) of hippocampus). Other sites were the cortex, cerebellum, anterior commissure and olfactory bulb. Histomorphometric values in different brain regions in adults, juveniles and neonates include maximum cortex width (19.27±0.2mm, 20.67±0.2mm, and 11.75±0.6mm) and cortex volume (2.84±0.0cm^3, 3.16±0.1cm^3 and 0.23±0.0cm^3) respectively. Ki-67 cell count in the SVZ and DG respectively were also highest in juveniles (45530±13950 and 12480±7860) as against adults (6880±340 and 1130±150) and neonates (21145±8395 and 11800±1230).

Findings in the olfactory bulb, multipolar neurons and the rare occurrence of myelination across all olfactory bulb layers indicated enhanced olfactory acumen. Increased receptive fields and synaptic glomerular surface area is also indicative of greater integrative computational capabilities. The juvenile African giant rat is clearly shown to have a greater capacity for neuroplasticity and thus, proposed as a model for rodent studies on olfaction and cognition.

Keywords: Neurocellular profile, Neurogenesis, *Cricetomys gambianus*, Neuroplasticity

Word Count: 441

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DEDICATION

This project is dedicated to the glory of God, my wife and three sons who are the best mirrors of God's love to me daily, here on earth. I'm eternally grateful.

I dedicate this work again in loving remembrance of Olatubosun AnjolaOluwa (1977-2010) and Titilope Irebowale Agbato (1980-2013). You were beautiful in your lives; sleep on in the bossom of your creator till we meet to part no more.

"In such difficult fields, however, the full truth rarely emerges at one strike. It is pierced together, little by little, through many trials and corrections"

Santiago Ramon *y* Cajal 1852-1934
Recollections of my life

CERTIFICATION

I hereby certify that this research was carried out by Olude, Matthew Ayokunle, Matric. number 90513 under my supervision.

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LIST OF ABBREVIATIONS AND DEFINITIONS

ABC	Avidin Biotin Complex
AGR	African giant rat
AHN	Adult Hippocampal neurogenesis
ANOVA	Analysis of variance
ANT COMM	Anterior commisure
DCX	Double cortin
CNPase	2’3’ cyclic nucleotide phosphodiesterase
GFAP	Glia fibrillary acidic protein
IbA-1	ionizing calcium binding adapter molecule 1
AOB	Accessory olfactory bulb
A-P	Antero-posterior
APC	Adenomatous Polyposis coli
APOPO	Anti-PersoonsmijnenOntmijnende Product Ontwikkeling: "Anti-Personnel Landmines Detection Product Development" in English
BDNF	Brain-derived neurotrophic factor
BMP	Bone morphogenetic protein
BrdU	Bromodeoxyuridine
BSA	Bovine Serum Albumin
CDC	Center for Disease control
CEREB. GRAN	Granule cell layer of the cerebrallum
CEREB. PCL	Purkinje cell layer of cerebellum
CEREB. WM	White matter of cerebellum
CEREB.MOLE	Molecular layer of cerebellum
CNPase	Cyclic nucleotide 2, 3 phosphodiesterase
CNS	Central nervous system
DAB	Deoxyaminobenzoic acid
DCX	Double cortin
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor

EGL	External granular layer
EPL	External plexiform layer
EPLi	External plexiform layer (inner)
EPLo	External plexiform layer (outer)
ES	Embryonic stem
FDA	Food and drug administration
FGF2	Fibroblast growth factor 2
GABA	Gamma amino butyric acid
GCL	Granule cell layer
GFAP	Glia Fibrillary Acidic Protein
GFP	Green fluorescence protein
GL	Glomerular layer
H&E	Heamatoxylin and Eosin
H_2O_2	Hydrgoen peroxide
HIPP	Hippocampus
IbA-1	Ionizing Calcium binding adapter molecule 1
IPCs	Intermediate progenitor cells
IUCN	International Union for Conservation of Nature
K and B	Kluver and Barrera
Lifr+/	Leukemia inhibitory factor receptor
LINE-1	Long interspersed nuclear element-1
LOB	Left olfactory bulb
MAM	Methylazoxymethanol acetate
Max	Maximum
MBD1	Methyl-CpG binding protein 1
MCL	Mitral cell layer
ML	Molecular Layer
MOB	Main bulb olfactory
NCAM	Neural cell adhesion molecule
NKCC1	chloride ion channel
NRbS	Normal rabbit serum

NSCs	Neural stem cells
OB	Olfactory bulb
ONL	Olfactory nerve layer
PB	Phosphate Buffer
PBS	Phosphate-buffered saline
PC	Purkinje cells
PCL	Purkinje cell layer
PFA	Paraformalaldehyde
PL	Plexiform layer
POC	Piriform olfactory cortex
PSA-NCAM	Polysialylated neural adhesion molecule
PVL	Periventricular layer
rec.	Recessus
RGCs	Radial glial cells
RMS	Rostral Migratory Stream
ROB	Right olfactory bulb
SD	Standard deviation
SEM	Standard error of mean
SGZ	Subgranular zone
SNPs	Short neural precursors
SPSS 16	Statistical package for social sciences- version 16
Std err	Standard error
SVB / SVZ	Subventricular zone
TB	Tris base
TBS	Tris-Base Saline
TBST	Tris- buffer saline triton-X
TBST	Tris-Base Saline in Triton
VEGF	Vascular endothelial growth factor
VZ	Ventricular zone

CHAPTER ONE

1.0 INTRODUCTION

Rodents make up the Order rodentia which is the largest mammalian order with approximately 2016 species in 28 families including Squirrels, Beavers, Chipmunks, Gophers, Rats, Mice, Lemmings, Gerbils, Porcupines, Cavies, and the Capybara, with about (50%) fifty percent of the species of living mammals being rodents (Sheets, 1989). Typical rodent features include two incisors in each jaw; short forelimbs for manipulating food; and cheek pouches for storing food (Myers *et. al.*, 2006).

The African giant rat, also known as pouched rat is one of Africa's largest rodents. Two species have been distinguished: *Cricetomys gambianus*, which lives chiefly in Savannahs and around the regions of forests and human settlements; and *Cricetomys emini*, which occur mainly in rain forests. They are found in Central Africa, in regions south of the Sahara desert as far south as Zululand. This includes countries such as Nigeria among others where they are often incorrectly called rabbits or Nigerian rabbits (Ajayi, *et al.*, 1978; Happlod, 1987). In the indigenous African population, these rats are considered a delicacy and are often hunted for food (Ajayi, 1977a). They are known to be high in protein and are savoured in West Africa and it is fast becoming a mammal of increasingly diverse interest (Ajayi *et al.*, 1978) and in traditional fetish uses (Sodeinde and Soewu, 1999).

The growing importance of the African giant rat in Nigeria is captured in recent biomedical research on the haematology **(**Olayemi, *et. al.*, 2001), Helminth and Parasitic studies, surveys and disease epidemiology (Dipeolu and Ajayi, 1976; Bobe and Mabela, 1997), behavioural changes and domestication (Ajayi, 1974; Ajayi *et al.*, 1978). Also, there has been increasing investigations into the anatomy of these rats mainly in the area of reproduction e.g. male reproductive studies (Oke, 1988; Oke and Aire, 1989**;** Adeyemo and Oke, 1990**;** Oke and Aire, 1995), study of the oestrous cycle in the female African giant rat (Olowookorun, 1979; Oke and Oke, 1999) and ultra structural studies of the female reproductive organs (Akinloye, 2009). Preliminary work has been done on the morphometry of the gastro intestinal tract (Ali, *et al.*, 2008**)**, of the cerebellum and the fore brain (Nzalak, 2005, Ibe *et. al* 2010, 2011), the neurocraniometry and the bony skeleton (Olude, 2009; Olude *et al.*, 2009a; 2009b and 2010a, 2010b, 2011, 2013), These information on the animal may be required to domesticate and adopt the African Giant Rat (AGR) as a convenient laboratory animal for investigating diseases of

great significance to man and his domestic animals (Weinstein, *et al.*, 1992, Fjellanger 2003, Weetjens *et. al*., 2009).

1.1 JUSTIFICATION

The AGR has become a research animal in Nigeria arousing international interest but still lacking a lot in basic research information and on the cellular neuroanatomy in particular. Increased information in this field will be useful in describing the functional anatomy of this rodent to explain basic behavioural characteristics and patterns especially those of translational importance. Furthermore, research has been limited to gross morphometric studies and investigative techniques have not utilized advanced research techniques such as immunohistochemistry used in this study.

1.2 STUDY HYPOTHESIS AND RESEARCH QUESTIONS

The brain controls neural and behavioural patterns of the AGR (e.g. olfaction, cognition, limb dexterity). Therefore, what are the unique structures in the neurocellular profile that influence these patterns?

Adult neurogenesis has been identified to play a role in spatial memory and cognition. Therefore, what is the profile, pattern and possible functions of adult neurogenesis in the AGR?

1.3 GENERAL OBJECTIVES

To describe the brain of the African giant rat across age groups using gross and histological and histochemical methods (HandE, Nissl, Kluver and Barrera, Golgi Silver impregnation) techniques.

1.4 SPECIFIC OBJECTIVES

1. To localize and describe the anatomy of the basic cells in the brain of the AGR using immunohistochemical stains (GFAP, CNPase, IbA-1).
2. To determine the occurrence and pattern of neurogenesis in the brain of the AGR using immunohistochemical methods (Ki-67 and DCX antibody staining).
3. To measure basic morphometric parameters of the brain structure of the AGR across age groups and their functional significance to their behavioural patterns.
4. To relate the cytoarchitecture and neurogenesis in regions of the brain of the African giant rat to key behavioural patterns such as olfaction, memory and cognitive functions

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 TAXONOMY

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Rodentia

Sub Order: Myomorpha

Superfamily: Muroidea

Family: Nesomyidae

Subfamily: Cricetomyinae

Genus: Cricetomys

Species: *Cricetomys gambianus*

Binomial name: *Cricetomys gambianus*, Waterhouse 1840 (Happlod, 1987).

2.1.1 CURRENT TAXONOMIC TRENDS IN AGR CLASSIFICATION

Recent publications on taxonomy of the African giant rat indicate the presence of more than two species to include

Cricetomys gambianus

Cricetomys emini

Cricetomys kivuensis

Cricetomys ansorgei

Cricetomys sp1

Cricetomys sp2

Cricetomys sp3

The distribution of the AGR (genus *Cricetomys*) spans almost the whole of sub-Saharan Africa, stretching from the savannah zone of West Africa through the Guineo-Congolian forest block to the savannahs of East and southern Africa (Musser and Carleton, 2005). In what can be regarded as a most authoritative reference and checklist for mammals, Musser and Carleton (2005) recognized four species of giant pouched rat: *Cricetomys gambianus*, *Cricetomys emini*, *Cricetomys ansorgei*, and *Cricetomys kivuensis*. Before this, most influential publications such as Genest-Villard (1967), Rosevear (1969), and Kingdon (1997), although noting the presence of several forms across the geographical range of *Cricetomys*, recognized only two species. The first of these is the broad-snouted *C. gambianus*, which is spread across the savannahs of Africa and possesses a whitish-grey belly that is rather indistinctly defined in relation to the flanks. The second is the slim-snouted *C. emini*, occupying the Guineo-Congolian forest block and possessing a distinct white belly. Even subsequent to the publication of Musser and Carleton (2005), several publications (e.g. Perry *et. al*., 2006; Peterson *et. al*., 2006) have continued to recognize *C. gambianus* and *C. emini* as the only two species contained within the genus *Cricetomys*. This point of view has been retained in the most recent International Union for Conservation of Nature (IUCN) red list of threatened species (Olayemi *et al.*, 2012).

The statuses of the species no longer recognized have been repeatedly questioned. These include two of the taxa described as full species by Musser and Carleton (2005): *C. ansorgei*, Thomas (1904) sometimes thought to represent savannah-dwelling populations of *C. gambianus* in East and southern Africa, and *C. kivuensis,* sometimes thought to be a montane population of *C. emini*, in the Democratic Republic of Congo (DRC). Approximate distribution patterns of various *Cricetomys* species are named (Genest-Villard, 1967; Musser and Carleton 2005). These studies used phenotypic characters such as snout width; broad versus narrower snouts (Genest-Villard, 1967; Olayemi and Akinpelu, 2008), pelage colour (indistinctly white versus. distinct white bellies; Genest-Villard, 1967; Rosevear, 1969; Happlod, 1987), and ecology; savannah versus forest-dwelling populations (Genest-Villard, 1967). Each of these approaches has resulted in identifications that display considerable overlap for the features considered. As such, species distinctions based on morphological features only are rendered difficult and designated taxonomic statuses of all the *Cricetomys* species remain to be further explored (Musser and Carleton, 2005).

Recent research efforts employing molecular (cyt *b*) and multivariate craniometric approach on specimens collected across key localities in Africa spanning much of the range of *Cricetomys* to provide further insight into the systematics of this genus, and to elucidate the taxonomic statuses and relationships of many populations of *Cricetomys* that currently lie in doubt, has identified the presence of three out of the four species currently recognized under the genus *Cricetomys*, i.e. *C. gambianus*,*C. ansorgei*, and *C. emini*, along with the unexpected presence of three additional so-far undescribed species, *Cricetomys* sp. 1 (forest zone, West Africa), *Cricetomys* sp. 2 (forest zone of Central Africa on the left bank of the Congo river), and *Cricetomys* sp. 3 (forest zone of Central Africa, right bank of the Congo River in Cameroon, Gabon, and the Republic of Congo) (Olayemi *et al.*, 2012). *Cricetomys kivuensis* is the only species recognized in the latest rodent checklist (Musser and Carleton, 2005) that was not detected in the study. This shows that there are at least three more taxa within the genus *Cricetomys* whose formal description will require a detailed comparison with all existing relevant types.

Several publications, employing alternative techniques such as karyotyping (Granjon *et al*., 1992; Codjia *et al*., 1994; Dobigny *et al*., 2002; Corti *et al*., 2005), plasma biochemical properties (Nssien *et al*., 2002), multivariate craniometry (Olayemi *et al.*, 2012), and the anatomy of the gastrointestinal tract (Ali *et al*., 2008), have attempted to provide additional information useful for characterization of the various giant pouched rat species. However, the taxonomic impact of these studies has been of restricted importance because they were conducted on limited specimen collections, underscoring the need for more investigations covering the entire range of these rodents. Peterson *et al.*, (2006) recommended the use of molecular methods to provide more insight into the taxonomy and phylogeny of Cricetomys. Preliminary molecular studies involving this genus have helped to clarify its position and relationships with regard to other groups within the rodent superfamily Muroidea. Until recently Cricetomys, based on dental morphology, was grouped alternatively under the family Muridae by authors who viewed its cheek teeth as triserial (Thomas, 1904; Ellerman, 1941; Simpson, 1945; Roberts, 1951), or under the family Cricetidae by those who considered its cheek teeth to be biserial (Petter, 1966; Rosevear, 1969; Reig, 1980, 1981). Molecular techniques, however, have established this genus and others within the subfamily Cricetomyinae as close relatives of archaic African muroids such as the Nesomyinae, Dendromurinae, and Mystromyinae (DuBois

et al., 1996), and hence belonging to the family Nesomyidae as earlier proposed by Lavocat (1973, 1978:Chaline *et al.*,1977).

2.1.2 THE ZYGOMASSETERIC SYSTEM

The zygomasseteric system (or zygomasseteric structure) in rodent is the anatomical arrangement of the masseter muscle of the jaw and the zygomatic arch of the skull. The anteroposterior or propalinal (front-to-back) motion of the rodent jaw is enabled by an extension of the zygomatic arch and the division of the masseter into three distinct parts.

The main types are described as protrogomorphous, sciuromorphous, hystricomorphous, and myomorphous.

2.1.2.1 Protrogomorphy

This is the primitive condition, found in the family Aplodontiidae and in various fossil groups. The rostrum is unmodified and the masseter originates on the ventral zygomatic surface. The Bathyergidae is considered secondarily protrogomorphous since its zygomatic condition clearly derived from a hystricomorphous ancestor (Bell, 2004).

2.1.2.2 Sciuromorphy

The ventral surface of the zygoma tilts and broadens into a zygomatic plate. The masseter lateralis extends forwards onto the rostrum. The masseter superficialis extends forwards along the zygoma. This condition is found in most of the family Sciuridae (suborder Sciuromorpha) and also in the Castoridae, the Eomyidae, and the Geomyidae (Bell, 2004).

2.1.2.3 Hystricomorphy

The masseter medialis is enlarged, and passes through an enlarged infraorbital foramen to originate on the side of the rostrum. The masseter superficialis originates on the front edge of the zygoma, and the masseter lateralis extends over most of its length. This condition is found throughout the suborders Hystricomorpha and Anomaluromorpha. In the suborder Myomorpha,it is found in the superfamily Dipodoidea and some fossil Muroidea (such as *Pappocricetodon*).

Hystricomorphy is also found in the African dormouse*Graphiurus*, which is a member of the suborder Sciuromorpha (Bell, 2004).

2.1.2.4 Myomorphy

It is a combination of the expanded and tilted zygoma of sciuromorphy and the enlarged foramen of hystricomorphy. This condition is found in the Muroidea (Myomorpha) and most Gliridae (Sciuromorpha: in the latter it is often referred to as *pseudomyomorphy*). Korth and Emry (1991) suggest that the infraorbital foramen of the extinct sciurid subfamily Cedromurinae may have allowed for the passage of the masseter muscle. If true, this subfamily would represent an additional example of myomorphy in the rodent suborder Sciuromorpha (Bell, 2004). Tullberg (1899) broadly categorized rodents into two distinct groups which he called sciurognathous and hystricognathous on the basis of jaw morphology. When viewed ventrally, the angle of the jaw in sciurognathous rodents arises in the plane of the incisor, whereas in hystricognathous rodents the angle arises lateral to the plane of the incisor. The Sciuridae, squirrels, are clear examples of sciurognathous morphology while the Hystricidae, Old World porcupines, are good examples of the hystricognathous condition (Dempsey, 1991).

Other defining characteristics of members of the order Rodentia are a brain with few convolutions; a cerebellum that is not covered by the cerebral lobes; a wide diastema between the incisors and cheek teeth into which the cheeks can extend and meet in the midline, thus separating the rostral and caudal portions of the mouth; four cheek teeth per side in upper and lower jaws; well-developed masseters; the presence of clavicles; a large caecum; and the fact that they are generally pentadactylous and plantigrade (Feldhamer *et al.,* 1999).

2.1.3 FAMILY- NESOMYIDAE

The Nesomyids are classified in 5 subfamilies, 22 genera and 55 species (Steppan *et. al.*, 2004).

Cricetomyinae (pouched rats)

Delanymyinae (Delany's Mouse)

Dendromurinae (climbing mice)

Mystromyinae (white-tailed rat)

Nesomyinae (Malagasy rats and mice)

Petromyscinae (African rock mice)

2.1.4 SUBFAMILY: CRICETOMYINAE

The subfamily Cricetomyinae contains three genera and five species.

Subfamily Cricetomyinae - Pouched rats

Genus *Beamys* - Lesser pouched rat

Beamys hindei - Lesser pouched rat

Genus *Cricetomys* - Giant pouched rats

Cricetomys gambianus - Gambian pouched rat

Cricetomys emini - Emin's pouched rat

Genus *Saccostomus* - Pouched mice

Saccostomus campestris

Saccostomus mearnsi

2.1.5 GENUS: CRICETOMYS

Giant pouched rats are only distantly related to the true rats, but are instead part of an ancient radiation of African and Malagasy muroids in the family Nesomyidae. They are named due to their large cheek pouches. There are two major species i.e. the *C. gambianus, and C. emini* (Nowak, 1999).

2.2 PHYLOGENY

Rodent phylogeny and classification has been the focus of many disagreements, some of which continue. Current hypothesis about the phylogeny of rodents, however, suggested that the difference in pattern of insertion of the masseter (sciurognathy versus hystricognathy) reflected a deep phylogenetic division (Myers *et al.,* 2006**).**

Paleontologists and mammalogists have successfully established relationships among rodents with study and analysis of cranial foramina (Wahlert *et al.*, 1993). Foramina are the holes that penetrate bones and allow passage of nerves, arteries, and veins through them. The skull is especially perforated with foramina since it houses the brain, and nerves and vessels must pass through bone to reach the eye socket and sensory hairs on the side of the snout. The positions of cranial foramina relative to one another, to the dentition, and to the sutures where bones varied; Much of these variations are consistent within related species and can be used to provide new data for analysis of relationships. The cranial foramina are especially important since they belong to systems that are mostly independent of the masseter musculature and dental characteristics on which hypotheses of interrelationship are traditionally based (Dempsey, 1991).

2.3 NATURAL HISTORY

The Gambian pouch rat (*Cricetomys gambianus*), also known as the African giant pouch rat is a nocturnal rat. Giant pouched rats are only distantly related to the true rats, but are instead part of an ancient radiation of African and Malagasy muroids in the family Nesomyidae. They are named due to their large cheek pouches. Research has shown that the cheek pouches of *C. gambianus* most likely evolved in parallel to those of Sciuridae and other members of the family Muridae (Ryan, 1989).It has been suggested that females may be capable of producing up to 10 litters yearly, with offsprings numbering 1-5 **(**Myers *et al*., 2006**)** after a gestation of 27-36 days.

The animals are omnivorous and feed on vegetation and invertebrates. They have a particular taste for palm nuts and have been described to be coprophagous. They can become tame and are kept as pets and are also an important food source in many African countries (Myers *et al.*, 2006).

2.4 HABITAT

In its native Africa, this rat lives in colonies of up to twenty, usually in forests and thickets, but also found in termite mounds (Wikipedia, 2008). Gambian rats inhabit a variety of habitats ranging from arid to temperate areas, but need some form of shelter to survive. They are therefore not usually found in completely open areas, but in areas with cover ranging from hollow trees, rock outcroppings, or burrows made by other animals. They are occasionally known to venture into urban areas and can become pest animals (Ajayi, 1977a). Although Gambian rats are usually passive and shy in the wild, they are very protective of their nests and are aggressive in defending it. However, outside of the nest, there is no truly defined home range (Ajayi, *et al.*, 1978**;** Nowak and Paradiso, 1983).

2.5 PHYSICAL DESCRIPTION

The head and body length of the African giant rat ranges from 25-45cm (9-18 inches) with scaly tails ranging from 36-46 cm (14-18 inches). Females weigh between 1-1.5 kg and males have been known to reach nearly 3 kg (2.2-6.5 lbs) and an average basal Metabolic Rate of 71 cm^3 oxygen/hour (Ajayi, 1977b; Ryan, 1989; Nowak and Paradiso, 1983)

The main physical characteristic of Gambian rats and all Cricetomys in general are their large cheek pouches. These pouches can expand to a great size, allowing them to transport massive quantities of food if necessary. Cheek pouches also exist in other families of rodentia, such as the African hamster and members of the subfamily Cricetinae. The females have eight nipples which are 4 in the thoracic region and 4 in the inguinal region (Akinloye, 2009).These rats also have a very low fat content, which may be the cause of their susceptibility to cold (Ajayi, 1977b).

2.6 LIFESPAN AND LONGEVITY

Gambian rats live for about 5 to 7 years in captivity, although some have been known to live as long as 8 years. Life expectancy in the wild is hard to document because of the small size of these creatures and because they are hunted so often by indigenous people (Ajayi *et al.*, 1978; Nowak and Paradiso, 1983). These rats adapt rapidly to new situations, such as captivity. (Ajayi, 1977a; Ajayi *et al.*, 1978).

2.7 BEHAVIOUR

Gambian rats are nocturnal animals, mostly due to the fact that they have little or no tolerance for the intense heat of a typical African day. They collapse readily under the hot Sun of Africa. Thus, they are nearly inactive during the day, and come out at night in search of food. Local hunters describe a vast system of tunnels or hollow trees used by the Gambian rats for their nests, where they rest during the day and come out at night in search of food (Nowak and Paradiso, 1983, Knight, 1988**)**. These nests are often located in cool areas, providing more evidence for their intolerance to heat. Interestingly, Gambian rats find almost as much value in the act of carrying as much as the act of hoarding food (Ajayi, 1977a; Ajayi *et al.*, 1978). This results in confusing hoarding patterns when food is plentiful in any season. The pouches inside the cheeks of Gambian rats can hold over 100 ml when full and this allows Gambian rats to transport an extraordinary amount of material in a short period of time. Some studies have shown Gambian rats to transport 3 kg in two and a half hours (Ajayi, 1977a; Ajayi *et al.*, 1978). The rats are also very good climbers and swimmers, and climb in excess of 2 meters easily. Both sexes are very territorial. Although Gambian rats are generally solitary in the wild, females often form large groups containing many mothers and their litters while males usually remain solitary. Gambian rats have also been known to huddle together when temperatures drop. Due to their low body fat they do not retain heat easily (Ajayi, 1977a; Ajayi *et al.*, 1978; Nowak and Paradiso, 1983).

2.8 FOOD HABITS

African giant rats are omnivores and feed on a variety of fruits, vegetables, nuts, and even insects when available. Some common foods include cassava, beans, sweet potatoes, and other roots. Termites have been known to be eaten and also snails but, apparently preferring palm fruits and palm kernels (Ajayi, 1977a; Kingdon, 1989). The cheek pouches allow it to gather up

several kilograms of nuts per night for storage underground. It has been reported to stuff its pouches so full of date palm nuts so as to be hardly able to squeeze through the entrance of its burrow. The burrow consists of a long passage with side alleys and several chambers, one for sleeping and the others for storage (Wikipedia, 2008).

2.9 ECOSYSTEM ROLES

African giant rats serve to keep insect populations under control, but also act as dispersers of seeds from different plants when they eat the fruits produced (Ajayi, 1977a, 1977b). Several parasitic worms inhabit the gastrointestinal tracts of these rats, but the most prevelant of these are the Strongyloides. A study performed also revealed minor presences of tape worms among other parasites. Other parasites include *Xenopsylla cheopis, Aspicularis tetraptera, Ixodes rasus,* and *Ornithonyssus bacoti*. Hymenolepis is usually found in the small intestine while Aspicularis is found in the rectum and colon (Bobe and Mabela, 1997; Dipeolu and Ajayi, 1976).

2.10 DISTRIBUTION

Gambian rats are found in regions south of the Sahara desert as far south as Zululand. This includes countries such as central Africa, Nigeria among others (Ajayi, *et al.,* 1978; Kingdon, 1989). The rats inhabit a variety of habitats ranging from arid areas to temperate areas, but need some form of shelter to survive. Therefore, they are usually found in areas with cover from hollow trees, rock outcroppings, or burrows made by other animals (Ajayi, 1977a; Kingdon, 1989).

2.11 USES AND ECONOMIC ADVANTAGE

2.11.1 Economic Importance for Humans: Negative

Gambian rats are sometimes considered pests in urban areas where they may infest the sewers. In rural areas, they may destroy farm crops and build burrows in the soil which lead to soil desiccation and loss of plant crops. Gambian rats often inhabit barns and other farm buildings which can lead to property damage (Ajayi, 1977a). In 2003 these rats were termed as invasive species in some parts of Florida where it was feared that they may cause great ecological damage; compete for food with native species, carry diseases, and damage the bird population by eating their eggs (Perry, *et al.,* 2006; Peterson, *et al.,* 2006). Gambian rats also act as transporters of seeds from different plants when they eat the fruits produced (Ajayi, 1977a).

This African rodent is also believed to be responsible for the outbreak of monkey pox in the United States. In 2003, the United States' CDC (Center for Disease control) and the FDA (Food and drug administration) issued an order preventing the importation of the rodents following the first reported outbreak of monkey pox. Several African species are believed to carry the disease (Wikipedia 2008).

2.11.2 Importance for Humans: Positive

The biggest economic impact of Gambian rats is as a source of food in Africa. They are considered rather tasty and are hunted and even raised on farms for their meat, this had led to a significant drop in their population (Ajayi 1974). Their meat forms part of the bush meat trade and the smoked carcasses of these rodents are often sold in the villages and towns; attempts are being made in Nigeria and other countries in African to domesticate these animals in captivity for food and research (Ajayi, 1974).

A smaller industry is the pet industry, although these rats are rather large and sensitive to temperature changes, resulting in a need for high maintenance. More recently, the animal has been used to sniff out landmines in Mozambique (Lindow, 2001) and in the diagnosis of Tuberculosis (Fjellanger, 2003). In the scientific community, these rats are often used for experiments, and these rats provide a wealth of information on rodent physiology and behavior (Ajayi, 1977a; Kingdon, 1989).

Information gathered from local hunters revealed that traditionalists in Africa often times use the African giant rat as a part of their elements for traditional rituals (Sodeinde and Soewu, 1999).

2.12 THE APOPO PROJECT FOR LANDMINE DETECTION AND TUBERCULOSIS DIAGNOSIS

The African giant rat is a nocturnal animal and one of Africa's largest rodent with known important olfactory sensory system evidenced by a relatively large olfactory bulb and olfactory areas (Nzalak *et al*. 2005) and its utilization to detect landmines and tuberculosis in some parts of the world by the APOPO group (Verhagen *et al*.,2003; Weetjens *et al*.,2009). APOPO, an acronym for Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling: "Anti-Personnel Landmines Detection Product Development" in English, is a registered Belgian non-

governmental organization which trains African giant pouched rats to detect landmines and tuberculosis (Verhagen *et al*., 2003); Weetjens *et al*. 2009).

2.13 OVERVIEW OF RESEARCH ON THE AFRICAN GIANT RAT

Heamatological research has also been documented by various authors (Olayemi *et al.*, 2001). Survey of intestinal and blood parasites affecting the rats in the wild and possible public health implications (Ajayi, 1977a; Bobe and Mabela, 1997; Sambo *et al.*, 2008). Research on the African giant rat has been undertaken in the areas of taxonomy, domestication (Ajayi 1977a), reported case studies (Dipeolu *et al*., 1981; Olude *et al*., 2010), development and production studies involving studies of the Anatomy of the male and female reproductive tracts and its adaptation to breeding and domestication (Oke 1988; Oke and Oke 1999**;** Akinloye 2009).

2.14 REVIEW OF ANATOMICAL RESEARCH ON THE AFRICAN GIANT RAT

A total of seventy one(71) scholarly research topics and articles were found published from a combined search of pubmed and google scholar and library resource in the field of anatomy (see tables 1and 2) with reproduction having the greater share with 32.4% while myology, endocrinology and arthrology had no representation. Major advancements in this field began in the early 1980s with morphometric analysis of male accessory glands and other parts of the male reproductive organs including the bulbo urethral gland (Oke and Aire, 1989), prostate gland (Oke and Aire, 1995), vesicular gland (Oke and Aire, 1997), epididymis (Oke *et al*.1987, Oke, 1995) and the coagulating gland (Oke and Aire, 1996). Oke *et al*., (1987) divided the epididymis into 5 histological zones and described their structure and histochemical properties. Seasonal effect on the reproductive anatomy was also examined in males (Oke, 1985). It was not until 1989 that ultrastructural studies began with the work of Oke (1988); Oke *et al*., (1989); and Oke and Aire (1990) on the epididymis and other components of the male system. Two sub zones were further elucidated from zone 5 of the epididymis as a further work from pilot studies. They concluded that, the epididymis of the AGR was capable of synthesising abilities as shown by its highly developed endoplasmic reticulum content further corroborating the all year round sexually active status of this rodent. Anatomical investigations of the female reproductive tract commenced with vaginal cytology and seasonal changes (Oke and Oke, 1999; Oke *et al.*, 2000), morphometric and seasonal morphometric evaluations of the female tract (Akinloye and Oke, 2009b) which revealed the AGR as having a duplex uterus and two cervixes, 8 mammary glands

(4 in the lateral inguinal region and 4 in the lateral thoracic region arranged in cranial and caudal rows). Further studies include ultrastractural and hormonal patterns (Akinloye and Oke, 2009a). The most recent internationally recognised publications utilised immunohistochemistry to detect oestrogen, progesterone receptors (where the intensity was tagged to the seasonal variations) and smooth muscle activity in the ovary (Madekurozwa *et al.*, 2010); similarities were noted with wistar rats with some species specific dependency at various stages of the season (Selstam *et al.*, 1993; Callebaut and Van Nassauw, 1987). Ali *et al.*, (2010), utilised morphometry to generate data on the non-gravid reproductive organs of the AGR. Most recent paper looked at the effect of unilateral testicular orchidectomy on testosterone and testicular parameters (Duru *et al*., 2013).

Studies on the kidneys have demonstrated calbindin localization in AGR kidney (Moutairou *et al*., 1996). Other studies have been morphometric (Onyeanusi *et al*., 2007; 2009) who measured and compared the urinary system of the AGR and the wistar rat taking into account the sex differences and the functional morphology of the kidneys. The earliest published record of any article involving the AGR is the histochemical study of the adrenal cortex (Quenum and Camain 1959). The authors did several related works also on the adrenal cortex, determined the effects of castration on the adrenal cortex and the problem of compensatory hypertrophy of the adrenal cortex (Camain and Quenum, 1961, Quenum, 1962a; Quenum and Camain, 1962 and Quenum, 1962b).

In the field of Osteology, 9 (15.5%) publications were observed as at the time of this review with advancements in the study of the skull. Studies by Olayemi and Akinpelu (2008) and Olude *et al.*, (2009a) characterized and classified this rodent on the basis of skull morphometry and showed significant sexually dimorphic parameters in the skull. In addition, other neurocraniometric studies revealed shape variations in the occipital region particularly in the foramen magnum and in the multiplicity of the hypoglossal foramina; the authors also highlighted the possibility of the estimation of the brain density and the use of the African giant rat for cranial pressure experiments (Olude *et al.*, 2009b). Four parameters were statistically significant between both sexes including the intercondylar width, temporal bone height, external auditory pore height and the sub arcuate fossa height in skull typology studies (Olude and Olopade, 2010). Among the morphologic findings was the presence of a ventral mandibular foramen, a complete jugal arch and presence of jugal foramen. The appendicular skeleton has also been described (Olude *et al.*, 2009c: 2010) with similar studies by Salami *et al.*, (2011a,

b).The pelvic limb studies resulted in a copyright finding on the distal portion of the tibia hitherto not reported in literature which the authors named the tibial depression of Olude (Olude *et al.*, 2009c; Nigerian Copyright Commission notification number: CN/L/2560). The forelimb also revealed some prominent features in the forelimb bones of the AGR that typifies them as fast running, burrowing and shoveling rodents due to the presence of the supracondylar foramen which is typically observed only in cats and the well developed ridges and tuberosities which are better developed in all burrowing animals (Olude *et al.*, 2010). Morphometric studies of the bony orbit and ocular dimensions were also documented by Olude *et al.*, (2011) and the most recent being studies on the axial skeleton, ribs and sternum (Olude *et al.*, 2013).

Articles on the digestive system began with investigations on the micro stereological and histochemical studies (Asojo and Aire, 1983) describing the structure of the three salivary glands and comparing it with laboratory rodents. Knight M.H. who obtained a PhD in 1988 studying the digestive tract of the AGR had co-published an extensive pilot article on the morphological, anatomical and physiological aspects of the digestive tract of the AGR, to test the relevance of two hypotheses relating structure to dietary habits (Knight and Knight-Ellof, 1987). The authors stated that the gut as a whole appears intermediate on a scale from omnivory to herbivory. The unilocular stomach with its papillated corpus and fornix ventriculi and dense bacterial mass, important in starch, glucose and nitrate reduction, and the fully glandular antrum are not indicative of cellulose digestion. The long small intestine emphasizes its importance in the digestion and absorption of protein and other metabolites; while the rather voluminous, differentiated caecum, with its folds and ridges designed to direct and retard digesta flow, is suggested to have considerable nutritional value. The nutritional value of the AGR meat has been analysed to contain over 65% Moisture, 20% Crude protein, 11% Fat, 1% Carbohydrates and Ash making up the rest per 100grams of muscle meat (Oyarekua and Ketiku, 2010). The complex gut, with its characteristically long passage rates, is suggested to be a product of many selective forces. Paneth cell studies were done in the stomach and compared with the colon (Satoh *et al.*, 1994). Ali *et al.*, (2008) worked on the gross morphometric aspects of the gastrointestinal tract of the African giant rat comparing it to the wistar rat, dog, ruminants and equines. Morphometric studies also have been carried out on the accessory digestive organs (Nzalak *et al.*, 2010).

In neuroanatomy, earliest advancements also date back to the work of Quenum (1961;1962c) who studied the various cell components of the pituitary gland. This was later followed by investigations by Piechl and Moutairou (1998) who noted the absence of S-cones retinae of the two species of AGR using immunohistochemical methods. Bastianeli *et al.,* (1999) published their work on the pineal gland where they demonstrated the presence of true neurons in the rodent pineal gland by using immunohistochemistry with five antibodies against calcium-binding proteins (calbindin-D28k, calretinin, calmodulin, neurocalcin and S-100/3) and thus postulated their functions. Ever since then, other publications have focused on functional morphometric studies (Nzalak *et al.,* 2005; Ibe *et al.,* 2010a; 2014) and histochemical studies (Ibe *et al.,* 2011a) of different parts of the brain. Studies on the olfactory bulb structure and adult neurogenesis profile were most recently published (Olude *et al.,* 2014a; 2014b). This is in part driven by the quest for domestication and a much recent use of the African giant rat as a research model because of its sheer size, strong olfactory powers and big sized brain for neuroanatomical research.

The earliest published information on the respiratory system was by Valerius (1996) who worked on the comparative morphology and structural patterns in the conductive bronchial tree of four species of myomorph rodents of different body weights. This was determined by lung casts which were inflated to 20 cm H_20, frozen, freeze-dried, hardened, and filled with silicone rubber. The casts were pruned, and branching pattern, diameter, and volume of the conductive bronchial tree were determined using a binocular magnifier. The AGR in particular was described to have four lobes on the right lung and an undivided left lung, and the central bronchial tree on either side shows an identical monopodial branching pattern. The diameter of the left main bronchus equalled 0.6% of body length in *Cricetomys,* and the conductive bronchial tree made up 6% of the total lung volume. He concluded that relatively wider airways and a decline in airway resistance with declining body mass in small mammals compared to large ones resulted in a high ventilatory dead space, which is compensated for by a higher breathing frequency. Further work on the respiratory system has been on the vomeronasal organas present in the lateral wall and on the dorso-lateral region between the sensory and non-sensory epithelia; suggesting that the organ is important in sexual behaviours (Igbokwe and Nwaogu, 2009). Most recent publications were on microscopic and macroscopic anatomy of the lower respiratory system (Ibe *et al.,* 2011b, c).

Angiology was limited to blood studies (Oyewale *et. al*., 1998; Oke *et al.*, 2000; Olayemi *et al.*, 2001) and recently the spleen with conclusions that the high spleen size is indicative of effective blood conservation in compensation for their subterranean habitat characterised by low oxygen (Ibe *et al.*, 2010c).

Anaesthesiology was low in research with only one article published on the adaptive vibrissae (Ibe *et al.*, 2010b). Our search showed no form of publications in the fields of myology, endocrinology and arthrology**.**

Table 1: Broad classification of published articles on the AGR found in various fields of anatomy

Field of study	Published Article
Osteology	11
Myology	-
Arthrology	-
Angiology	8
Respiratory	4
Endocrine	-
Neuroanatomy	11
Reproduction	23
Urinary and Adrenals	8
Digestive	5
Anesthesiology	1
TOTAL	71

Table 2: Distribution pattern of publications on the AGR in the past six decades in various fields of anatomy

Field of study	1951-1960	1961-1970	1971-1980	1981-1990	1991-2000	2001-2010	2011-2014	Total	Percentage (%)
Reproduction	-	-	-	7	6	8	2	23	32.4
Digestive	-	-	-	2	1	2	-	5	7.0
Neuroanatomy	-	2	-	-	3	1	5	11	15.5
Osteology	-	-	-	-		6	5	11	15.5
Respiratory	-	-	-	-	1	1	2	4	5.6
Myology	-	-	-	-	-	-	-	-	-
Angiology	-	-	1	1	4	2	-	8	11.3
Urinary	1	4	-	-		3	-	8	11.3
Endocrinology			-	-	-	-			-
Anesthesiology	-	-	-	-	-	1	-	1	1.4
Arthrology	-	-	-	-	-	-	-	-	-
TOTAL	1 (1.4%)	6 (8.5%)	1 (1.4%)	10 (14.1%)	15 (21.1%)	24 (33.8%)	14 (19.7%)	71	100%

2.15 EMBRYOLOGY OF THE BRAIN

The central nervous system (CNS) appears as a slipper-shaped plate of thickened ectoderm, the neural plate, in the mid-dorsal region in front of the primitive node. Its lateral edges then elevate to form the neural folds. With further development, the neural folds continue to elevate, approach each other in the midline, and finally fuse, forming the neural tube (Gilbert, 2010).Fusion begins in the cervical region and proceeds in cephalic and caudal directions and once fusion is initiated, the open ends of the neural tube form the cranial and caudal neuropores that communicate with the overlying amniotic cavity. Closure of the cranial neuropore proceeds cranially from the initial closure site in the cervical region and from a site in the forebrain that forms later. This later site proceeds cranially, to close the rostral most region of the neural tube, and caudally to meet advancing closure from the cervical site. Final closure of the cranial neuropore occurs at the 18- to 20-somite stage while closure of the caudal neuropore occurs later (Nichols, 1981; Harrington *et al*., 2009).

The cephalic end of the neural tube showed three dilations, the primary brain vesicles: (*a*) the prosencephalon, or forebrain; (*b*) the mesencephalon, or midbrain; and (*c*) the rhombencephalon, or hindbrain. Simultaneously, it forms two flexures: (*a*) the cervical flexure at the junction of the hindbrain and the spinal cord and (*b*) the cephalic flexure in the midbrain region**.** The prosencephalon further developed into two parts: (*a*) the telencephalon, formed by a mid-portion and two lateral outpocketings, the primitive cerebral hemispheres, and (*b*) the diencephalon, characterized by outgrowth of the optic vesicles. A deep furrow, the rhombencephalic isthmus, separates the mesencephalon from the rhombencephalon. The rhombencephalon also developed into two parts: (*a*) the metencephalon, which later forms the pons and cerebellum, and (*b*) the myelencephalon (medulla oblongata). The boundary between these two portions is marked by the pontine flexure (Guthrie and Lumsden 1991; Lumsden, 2004).The lumen of the spinal cord, the central canal, is continuous with that of the brain vesicles. The cavity of the rhombencephalon is the fourth ventricle, that of the diencephalon is the third ventricle, and those of the cerebral hemispheres are the lateral ventricles. The lumen of the mesencephalon connects the third and fourth ventricles. In humans, this lumen becomes very narrow and is then known as the aqueduct of Sylvius. The lateral ventricles communicate with the third ventricle through the interventricular foramina of Monro **(**Gilbert, 2010**)**

2.16 HISTOLOGICAL DIFFERENTIATION

2.16.1 Stem Cells and Precursor Cells

The stem cells of the neocortex are originally generated by symmetrical division of the neural tube epithelium before neurogenesis (neuron formation) actually occurs. Initially, the proliferative layer of the cortex forms the ventricular zone (VZ). Shortly thereafter (around day 13), it divides to give rise to a subventricular zone (SVZ) directly outside it. Together, these zones form the germinal strata that generate the neuroblasts (neuronal precursor cells) that migrate into the cortical plate and form the layers of neurons. The VZ will form the lower (deeper) layers of neurons, while the SVB will give rise to those cells that form the upper layer of neurons (Frantz *et al*., 1994).

There are three major progenitor cells in the germinal strata: radial glial cells (RGCs), short neural precursors (SNPs), and intermediate progenitor cells (IPCs). RGCs (which are not really glia but tend to look like them) are thought to be stem cells, and they are found at the VZ. At each division, they generate another VZ cell and a more committed cell type. Interestingly, at each division, the cell receiving the "old" centriole (which contains different proteins than the newly made centriole) stays in the VZ, while the cell receiving the "young" centriole leaves to differentiate (Wang *et al*., 2009). The more committed cells can be either neuroblasts (which divide to generate neurons) or IPCs, which migrate to the SVZ, where they generate neuroblasts. The SNPs also appear to generate neuroblasts, but from the VZ. A single stem cell in the ventricular layer can give rise to neurons (and glial cells) in any of the cortical layers (Walsh and Cepko 1988).

2.16.2 Cortical Cell Migration

The first cortical neurons to be generated migrate out of the germinal zone to form the transient preplate (Kawauchi and Hoshino 2008). Subsequently generated neurons migrate into the preplate and separate it into two layers: the Cajal-Retzius layer and the subplate. The Cajal-Retzius layer becomes and remains the most superficial layer of the neocortex, and its cells express the cell surface glycoprotein Reelin. The subplate remains the deepest layer through which the successive waves of neuroblasts travel to form the cortical plate. The Reelin-producing cells of the Cajal-Retzius layer are critical in the separation of the preplate. In Reelin-deficient mice, the preplate fails to split, and the neurons produced by the germinal layers pile up behind

the previously generated neurons (instead of migrating through them). By activating the Notch pathway, Reelin on the surface of the Cajal-Retzius cells allow the neuronal stem cell to produce a long fiber that extends through the cortical plate (Nomura *et al*., 2008). This (and the fact that it produced some proteins thought to be glial-specific) caused the neural stem cell to be called the radial glial cell. The process from this cell becomes critical for the migration of the neural cells produced by the germinal zones.

There are mutations that specifically affect the microtubular cytoskeleton of the migrating neuroblasts. Mutations in the DISC1 gene prevent neuronal migration in the cortex by interfering with microtubule assembly; humans with such mutations have been seen to suffer from mental dysfunctions, among them autism, bipolar diso, and schizophrenia (Gilbert, 2010).

Hanashina *et al.,* (2004) have shown that there are several genetic switches that get "thrown" at these division times. One of these switches is the gene encoding the transcription factor Foxg1. When the mouse neuronal progenitor cells divide to form the first layer of cortical neurons, Foxg1 is not expressed in the progenitor cells or in the first-formed neurons. However, later, when the progenitor cells generate those neurons destined for layers 4 and 5, they express this gene. If the Foxg1 gene is conditionally knocked out of this lineage, the neural precursor cells continually give rise to layer 1 neuron. Therefore, it seems that the Foxg1 transcription factor is required to suppress the "layer 1" neural fate.

Neither the vertical nor the horizontal organization of the cerebral cortex is clonally specified – that is none of the functional units form from the progeny of a single cell. Rather, the developing cortex forms from the mixing of cells derived from the numerous stem cells. The early regionalization of the neocortex is thought to be organized by paracrine factors secreted by the epidermis and neural crest cells at the margins of the developing brain (Rakic *et al*., 2009). The paracrine factors induce the expression of transcription factors in the specific brain regions, which then mediate the survival, differentiation, proliferation, and migration of the newly generated neurons.

For instance, Fgf8 protein is secreted by the anterior neural ridge and is important for specifying the telencephalon. If Fgf8 is overexpressed in the ridge, specification of the telencephalon is extended caudally, whereas if Fgf8 is ectopically added to the caudal region of

the cortex, part of that caudal region will become anterior (Fukuchi-Shimogori and Grove, 2001, 2005). Sonic hegdedog is secreted by the medial ganglionic eminence and helps form the ventral neurons of the cortex, including those of the substantia nigra (whose absence causes Parkinson disease).

2.16.3 Neurons to Glia

The stem cells of the vertebrate cortex make neurons first and then make glial cells. In mice, neurons are formed from embryonic day 12 through embryonic day 18. Then, at embryonic day 18, the same precursor cells generate glia. When cortical progenitor cells (cortical stem cells such as RGCs and their more committed desendants) are cultured on embryonic day 12, they differentiate into neurons for the first few days. After multiple days of culture, the progenitors switch and start making glia (Gotz and Barde, 2005). Retroviral tracers and time-lapse micro cinematography have shown that individual precursor cells make both neurons and glia (Reid *et al*., 1995; Qian *et al*., 2000). Both internal and external factors regulate this transition. Cortical precursor cells form neurons when cultured on embryonic cortical slices, but they become glia when placed on older cortical slices (Morrow *et al*., 2001). One of the main factors involved in this environmental regulation is CT-1, a paracrine factor that activates the JAK-STAT pathway. The STAT transcription factor activates glial-specific genes. Moreover, this transition must be done in the absence of neurotrophic factors, as these activate the RTK-MAPK pathway, resulting in neural-specific gene expression (Miller and Gauthier 2007).

2.16.4 Nerve Cells

Neuroblasts, or primitive nerve cells, arise exclusively by division of the neuroepithelial cells. The neuroepithelial cells give rise to three types of cells. First, they become ventricular (ependymal) cells that remain integral components of the neural tube lining and that secrete the cerebrospinal fluid. Second, they generate the precursors of neurons and third the glia precursors and their fate largely determined by their environment (Rakic and Goldman, 1981; Turner and Cepko, 1987; Gilbert, 2010). Initially they have a central process extending to the lumen (transient dendrite), but when they migrate into the mantle layer, this process disappears, and neuroblasts are temporarily round and apolar. With further differentiation, two new cytoplasmic processes appear on opposite sides of the cell body, forming a bipolar neuroblast. The process at one end of the cell elongates rapidly to form the primitive axon, and the process at the other end

shows a number of cytoplasmi arborizations, the primitive dendrites (Gilbert, 2010)**.** The cell is then known as a multipolar neuroblast and with further development becomes the adult nerve cell or neuron. Axons of neurons in the basal plate break through the marginal zone and become visible on the ventral aspect of the cord. Known collectively as the ventral motor root of the spinal nerve, they conduct motor impulses from the spinal cord to the muscles. Axons of neurons in the dorsal sensory horn (alar plate) behave differently from those in the ventral horn. They penetrate into the marginal layer of the cord, where they ascend to either higher or lower levels to form association neurons (Gilbert, 2010).

2.16.5 Glial Cells

The majority of primitive supporting cells, the gliablasts, are formed by neuroepithelial cells after production of neuroblasts ceases. Gliablasts migrate from the neuroepithelial layer to the mantle and marginal layers. In the mantle layer, they differentiate into protoplasmic astrocytes and fibrillar astrocytes. Another type of supporting cell possibly derived from gliablasts is the oligodendroglial cell**.** This cell, which is found primarily in the marginal layer, forms myelin sheaths around the ascending and descending axons in the marginal layer. In the second half of development, a third type of supporting cell, the microglial cell,appears in the CNS. This highly phagocytic cell type is derived from mesenchyme **(**Gilbert, 2010**).** When neuro-epithelial cells cease to produce neuroblasts and gliablasts, they differentiate into ependymal cells lining the central canal of the spinal cord. Neural Crest Cells During elevation of the neural plate, a group of cells appears along each edge (the crest) of the neural folds.

2.16.6 Cerebellar Organization

In the brain, cell migration, differential neuronal proliferation, and selective cell death produce modifications of the three-zone pattern seen in the cerebellum, some neuronal precursors enter the marginal zone to form nuclei (clusters of neurons). Each nucleus works as a functional unit, serving as a relay station between the outer layers of the cerebellum and other parts of the brain. Other neuronal precursors migrate away from the germinal neuroepithelium.

These cerebellar neuroblasts migrate to the outer surface of the developing cerebellum and form a new germinal zone, the external granular layer near the outer boundary of the neural

tube (Gilbert 2010). At the outer boundary of the external granular layer, which is 1-2 cells thick, neuroblasts proliferate and come into contact with cells that secrete the bone morphogenetic protein (BMP) factors. The BMPs specify the post-mitotic products of these neuroblasts to become a type of neuron called the granule cells (Alder *et al*., 1999). Granule cells migrate back towards the ventricular (ependymal) zone, where they produce a region called the internal granular layer. Meanwhile, the original ventricular zone of the cerebellum generates a wide variety of neurons and glial cells, including the distinctive and large purkinje neurons, the major cell type of the cerebellum.

Purkinje neurons secrete sonic hegdedog, which sustains the division of granule cell precursors in the external granular layer (Wallace, 1999). Each Purkinje neuron has an enormous dentritic arbor that spreads like a tree above a bulblike cell body. A typical purkinje neuron may form as many as 100,000 synapses with other neurons- more connections than any other type of neuron studied. Each purkinje neuron also sends out a slender axon, which connects to neuron in the deep cerebellar nuclei.. Purkinje neurons are critical in the electrical pathway of the cerebellum. All electrical impulse eventually regulate the activity of these neurons, which are the only output neurons of the cerebellar cortex. For this to happen, the proper cells must differentiate at the appropriate place and time.

For this to be accomplished, one mechanism thought to be important for the positioning of young neurons in the developing mammalian brain is glial guidance (Rakic 1972; Hatten 1990). Throughout the cortex, neurons are seen to ride a "glial monorail" to their respective destinations. In the cerebellum, the granule cell precursors travel on the long processes of the Bergmann glia (Gilbert, 2010; Rakic 1975). This neuro-glia interaction is a complex and fascinating series of events, involving reciprocal recognition between the glial and the neuroblasts (Hatten 1990; Komuro and Rakic 1992). The neuron maintains its adhesion to the glial cell through a number of proteins, one of them, an adhesion protein called astrotactin. If the astrotactin on a neuron is masked by antibodies to that protein, the neuron will fail to adhere to the glial processes (Edmondson *et al*., 1988; Fishell and Hatten 1991). Mice deficient in astrotactin have slow neuronal migration rates, abnormal purkinje cell development, and problems coordinating their balance (Adams *et al*., 2002). The direction of this migration appears

to be regulated by a complex series of events orchestrated by brain-derived neurophic factor (BDNF), a paracrine factor that is made by the internal granular layer (Gilbert, 2010).

2.16.7 Cerebral Cortex Development

The cerebral cortex develops from the pallium which has two regions: (*a*) the paleopallium, or archipallium, immediately lateral to the corpus striatum and (*b*) the neopallium, between the hippocampus and the paleopallium). In the neopallium, waves of neuroblasts migrate to a subpial position and then differentiate into fully mature neurons. When the next wave of neuroblasts arrives, they migrate through the earlier-formed layers of cells until they reach the subpial position. Hence the early-formed neuroblasts obtain a deep position in the cortex, while those formed later obtain a more superficial position. At birth the cortex has a stratified appearance due to differentiation of the cells in layers. The motor cortex contains a large number of pyramidal cells, and the sensory areas are characterized by granular cells.

2.16.8 Cerebral Cortex Organization

The three-zone arrangement of the neural tube is also modified in the cerebrum. The cerebrum is organized in two distinct ways. Like the cerebellum, it is organized vertically into layers that interact with one another. Certain neuroblasts from the mantle zone migrate on glial processes through the white matter to generate a second zone of neurons at the outer surface of the brain. This new layer of gray matter will become the neocortex. The specification of the neocortex is accomplished largely through the Lhx2 transcription factor, which activates numerous other cerebral genes. In Lhx2-deficient mice, the cerebral cortex fails to form. The neocortex eventually stratifies into six layers of neuronal cell bodies; the adult forms of these layers are not completed until the middle of childhood. Each layer of the neocortex differs from the others in its functional properties, the types of neurons found there, and the sets of connections they make. For instance, neurons in layer 4 receive their major input from the thalamus (a region that is from the diencephalon), whereas neurons in layer 6 send their major output back to the thalamus.

In addition to the six vertical layers, the cerebral cortex is organized horizontally into more than 40 regions that regulate anatomically and functionally distinct processes. For instance, neurons in layer 6 of the visual cortex projects axons to the lateral geniculate nucleus of the

thalamus, which is involved in vision, while layer 6 neurons of the auditory cortex (located more dorsal than the visual cortex) project axons to the medial geniculate nucleus of the thalamus, which functions in hearing.

One of the major questions in developmental neurobiology is whether the different functional regions of the cerebral cortex are already specified in the ventricular region, or if specification is accomplished much later by the synaptic connections between the regions. Evidence that specification is early (and that there might be some "proto-map" of the cerebral cortex) is suggested by certain human mutations that destroy the layering and functional abilities in only one part of the cortex, leaving the other regions intact (Gilbert, 2010).

Indeed, most of the neuroblasts generated in the ventricular zone migrate outward along radial glial processes to form the cortical plate at the outer surface of the brain. As in the rest of the brain, those neurons with the earliest birthdays form the layer closest to the ventricle. Subsequent neurons travel greater distances to form the more superficial layers of the cortex. This process forms an "inside-out" gradient of development (Rakic, 1974). McConnell and Kaznowski (1991) have shown that the determination of laminar identity (i.e., which layer a cell migrates to) is made during the final cell division. Newly generated neuronal precursors transplanted after this last division from young brains (where they would form layer 6) into older brains whose migratory neurons are forming layer 2 are committed to their fate, and migrate only to layer 6. However, if these cells are transplanted prior to their final division (during mid-S phase), they are uncommitted and can migrate to layer 2. The fates of neuronal precursors from older brains are more fixed. While the neuronal precursor cells formed early in development have the potential to become any neuron (at layers 2 or 6, for instance), later precursor cells give rise only to upper-level (layer 2) neurons (Frantz and McConnell, 1996). Once the cells arrive at their final destination, it is thought that they produce particular adhesion molecules that organize them together as brain nuclei (Matsunami and Takeichi, 1995).

According to Gaiano (2008), "the construction of the mammalian neocortex is perhaps the complex biological process that occurs in nature. A pool of seemingly homogeneous stem cells first undergoes proliferative expansion and diversification and then initiates the production of successive waves of neurons. As these neurons are generated, they take up residence in the nascent cortical plate where they integrate into the developing neocortical circuitry. The spatial

and temporal coordination of neuronal generation, migration, and differentiation is tightly regulated and of paramount importance to the creation of a mature brain capable of processing and reacting to sensory input from the environment and of conscious thought".

2.16.9 Olfactory Bulbs

Differentiation of the olfactory system is dependent upon epithelial-mesenchymal interactions. These occur between neural crest cells and ectoderm of the frontonasal prominence to form the olfactory placodes and between these same crest cells and the floor of the telencephalon to form the olfactory bulbs. Cells in the nasal placodes differentiate into primary sensory neurons of the nasal epithelium whose axons grow and make contact with secondary cneurons in the developing olfactory bulbs. By the seventh week, these contacts are well established. As growth of the brain continues, the olfactory bulbs and the olfactory tracts of the secondary neurons lengthen, and together they constitute the olfactory nerve (Gilbert, 2010).

2.17 BRAIN OF THE RAT (*Rattus norvegicus*)

The brain is that portion of the central nervous system contained within the skull. Anatomically, it is described based on the three primimtve embryonic divisions. The brain of the rat is thus, described to include the rhombencephalon, mesencephalon and prosencephalon

2.17.1 Rhombenecephalon

The medulla oblongata begins about 3mm rostral to the origin of the first cervical nerve (at the level of the foramen occipitale magnum) and extends to the pons. On its rounded vertical surface a shallow midline groove continues the median fissure of the spinal cord. It continues across the pons and bears the a. basilaris to the circulus arteriosus. Lateral to the median fissure lie the caudally flattened pyramids. At the end of the medulla oblongata they turn dorsally to enter the dorsal funiculus of the spinal cord. Rostrally the medulla oblongata is bounded by the transversely running band of the corpus trapezoideum.

The dorsal surface of the medulla makes up most of the floor (fossa rhomboidea) of the fourth ventricle. In its caudolateral region lie the eminentia vestibularis medialis. Laterally the medulla oblongata joins the cerebellum by means of the pedunculus cerebellaris caudalis. Caudal

and lateral to the peduncle the semilunar tuberculum acusticum (nuclei Cochlearis dors.) is found. Caudally the lateral walls of the ventricle merge at the right angle (calamus scriptorius) and support the area postrema. Further caudally the shallow the sulcus medianus dorsalis continues to the spinal cord. Laterally the fasciculus gracilis and cuneatus are found as indistinct, cranially diverging longitudinal striations.

On its ventral surface the pons is separated from the corpus trapezoideum and the pedunculi cerebri by shallow transverse grooves. Its lateral limits are at the exit of the roots of the trigeminal nerve; here it turns deeper as a connection with the cerebellum by means of the pedunculi cerebellares medii. On either side of the midline the course of the pyramidial tracts is visible beneath the superficial fibres of the pons.

The dorsal surface of the pons forms the rostral part of the fossa rhmboidea. The roof of the IV ventricle is formed by the cerebellum with its vela medullaria and in the caudal portion also by the tela chorioidea.

The cerebellum is, with two of its peduncles (caudal and middle) connected with the medulla oblongata and the pons; with its pedunculus rostralis, it is connected with the mesencephalon. It consists of vermis and the hemisphere, which display the lobular pattern typical for mammals. A characteristic of the rodent is the prominent paraflocculus. It consists of a lobules petrosus (paraflocculus accessorius), which together with the flocculus is located in the recess (fossa subarcuata) of the os petrosum and of a part outside of this fossa, the paraflocculus dorsalis. Both parts of the paraflocculus are connected with lobules VIII (pyramis) of the vermis by means of the copula pyramidis; there is also a narrow connection of lobules petrosus with lobules IX (uvula) (Brauer and Schober, 1970). The cerebellum of the African giant rat was observed to be globular, lying caudal to the cerebrum and dorsal to the fourth ventricles in the region of the Pons and rostral portion of the medulla oblongata. The dorsal view of the cerebellum revealed a wormlike appearance (Nzalak *et al.*, 2005).

2.17.2 Mesencephalon

The ventral surface of the mesencephalon bears the crura crebri, separated by the fossa cruralis (interpaduncularis). Dorsally the culliculi are very distinct; with the exception of the tips of the caudal culliculi they are covered from above by the occipital portion of the cerebrum, the pineal body, and the rostral part of the cerebellum.

2.17.3 Prosencephalon

The procencephalon consists of diencephalon and telencephalon. The parts of the diencephalon visible ventrally are the infundibulum of the pituitary gland and dorsal to it the tuber cinereum, which caudally merges with the corpus mammillare. Rostrally there lies the chiasma opticum. After removal of the cerebral hemisphere the thalamus with its corpus geniculatum mediale and laterale are visible dorsally.

In the telencephalon, the prominent development of the bulbus olfactorius and the absence of the gyri and sulci (lissencephalic brain) are conspicuous features. From the bulbus olfactricus, a white band, the tractus olfactorius lateralis, runs caudolaterally; medially it is attached to the tuberculum olfactorium. Lateral to the tractus the fissure rhinalis runs caudally, making up the borderline between isocortex and allcortex. The surface area of the entire cortex is 278.78mm^2; 129.04mm^2 of this area (or about 46%) belongs to the isocortex and 149.74mm^2 (or about 54%) to allocortex (Stephan, 1954); for the classification of the cortical area, (Krieg, 1946; and Vaz Ferreira, 1951) for weight and volume of the brain in different stages of life (Leibnitz, 1972).

The cerebral cortex is smooth (lissencephalic) rather than highly folded (gyrencephalic). An advantage of having a highly folded and wrinkled cortex is that the folds allow more gray matter per ounce of white matter (Cartmill, 1987; Nzalak *et al.,* 2005).). The rabbit and beaver are also lissencephalic mammals while the cat and fetal pig are gyrencephalic. This may mean that rats and other lissencephalic animals require fewer cell bodies (gray matter) than axons (white matter) (Cartmill, 1987) compared to gyrencephalic animals. The forebrain is the frontal division of the brain, which contains the right and left cerebral hemispheres, the thalamus, and the hypothalamus. The forebrain takes up more than half of the cerebral space, while the cerebellum and the olfactory bulbs each take approximately 25 percent of the space. The

olfactory bulbs are relatively gigantic compared to those of the cat, pig, opossum, and the rabbit. While the rabbit has large olfactory bulbs, they are much smaller relative to the rest of the brain and make up only about 12.5 percent of its brain space. The beaver is the only mammal in class that has olfactory bulbs comparable to those of the rat. The rat and the beaver, both in the order Rodentia, have poor vision and must rely on their olfactory and auditory senses in order to move around and orient themselves. Their poor vision is reflected by the size of their optic nerves, which are relatively small (Cartmill, 1987)

2.17.4 Ventricles of the Brain

In the region of the medulla oblongata the central canal of the spinal cord enlarges to form the fourth ventricle. Its floor is formed by the medulla oblongata and the pons, the lateral walls by the rostral and caudal cerebellar peduncle, the roof by the cerebellum and medullary vela. The caudal portion of the ventricle is narrow (about 1mm), the rostral part is enlarged (2.5mm). At the transition the paired recessus lateralis extends laterally; it communicates with the subarachnoidal space through the aperturae ventriculi. A lateral aperture (Luschkae) appears to be constant but a median aperture of the fourth ventricle is absent (Levinger, 1971). Dorsally the recessus dorsalis (fastigium) penetrates the cerebellum. Directly opposite, the floor of the ventricle is indented by a bipartite, short recessus incertus (Westergaard, 1969). At the transition to mesencephalon the ventricle narrows to form a 3mm long and 0.35-0.7mm wide aqueductus cerebri, which forms a centrally open curve. Dorsally from the vertex of the curve, arises the recessus colliculi caudalis (height 2.2mm). The third ventricle is a narrow midsagittal slit (width 0.5mm, height 5.0mm). It is interrupted in the centre by the massa intermedia (diameter 2.0mm) and therefore acquires a ring- shaped lumen. Its wider roof contains a wide, dorsally slanting recessus suprapinealis (height 1.0mm) and a shallow recessus pinealis. The floor of the third ventricle contains rostrally the short and pointed optic recess, caudallly the narrow recessus. infundibuli and a horizontally directed inframammillaris recess.

Above the rec. opticus the rostral wall of the third ventricle is indented by the commissural rostralis. The lateral walls of the ventricle are formed by the thalamus and hypothalamus. Dorsorostrally the slit- like interventricular foramina open into the lateral ventricles, which are the most voluminous part of the cerebral cavities (height 7mm). They are almost vertically oriented, slit-like space with a cornu rostrale located rostral to the foramen

interventriculare. The cornu has a pointed recessus olfactrius, extending a variable distance into the bulbus olfactrius (Böhme and Franz, 1967; Westergaard, 1969). The pars centralis of the lateral ventricle extends caudally and laterally from the foramen interventriculare. Ventrally it gives off almost transversally oriented cornu caudalis. Postnatally after degeneration of the ependyma, the walls of the pars centralis in the caudal region fuse in the form of circumscribed ridges. This process causes considerable change in the shape of the ventricle (Westergaard, 1969).

The ventricles are lined with a ciliated columnar ependyma. In the roof of third and fourth ventricles and the median wall of the lateral ventricle, the ependyma covering the capillary loops of the plexus chorioidei becomes cuboidal.

Special formations in the wall of the ventricular system are the subfornical organ, organon vasculosum, laminae terminalis, and area postrema and subcommissural organ.

The midline subfornical organ lies close to the interventricular foramen in the region of the corpus fornicis. The vascular organ lies between optic chiasma and rostral commissure. The area postrema is located above the opening of the central canal into the fourth ventricle. These three formations are circumscribed organs beneath a flattened ependyma. They consist of glial cells and special parenchymal cells (tanycytes) and contain numerous blood vessels and nerve (Cohrs, 1936; Weindl, 1965). The subcommissural organ is located rostral to the commissura caudalis in the roof of the third ventricle at the opening of the aqueductus cerebri. It consists of tall ependymal cells without basal processes. The ependyma rests on a glial tissue rich in blood vessel and cells, which in their straining properties resemble the subcommissural ependymal cells. They are often arranged in rosettes and are said to be secretory (Stanka, 1963).

From the surface of the ependyma arises Reissner's fibre, which can be traced throughout the whole central canal back to the filum terminale. Supposedly it is a secretory product of the subcommissural ependyma.

2.17.5 Meninges

The spinal cord and the roofs of the spinal nerves are closely surrounded by dura mater, a glassy, tough membrane. Only in the lumbar region is it pushed off the surface of the spinal cord by the dorsal and ventral roots.

Usually the roots penetrate the tube of dura mater through several closely fitting openings and each is accompanied by a dural sheath which is attached to the spinal ganglion as well as to the periosteum of the intervertebral foramen. The epidural space is traversed by sparse narrow blood vessels. Especially in the dorsal part of the thoracic and sacral region, it contains a 0.3-0.5mm layer of fat. In the cervical and lumbar region it is less than 0.3mm thick. The arachnoid, aclear membrane, is loosely but not displaceably connected with the thin pia mater.

Scattered projections, found in the thoracic region, represent the denticulate ligament (Waibl, 1973). Within the braincase, the dura mater, widely fused with the periosteum, penetrates deeply between the hemispheres and the cerebellum as falx cerebri and tentorium cerebelli. The richly vascularize pia mater lies close to the surface of the brain and spinal cord and fused with the adventitia of the penetrating blood vessels.

2.18 CRANIAL NERVES AND THEIR NUCLEI

Description of the cytoarchitectonics of the central nervous system will be restricted to the topography of the nuclei of the cranial nerves. For the topography of other nuclei and the tracts, see the special literature.

Olfactory nerve (I): As in other macrosinatic species the parts of the rhinencephalon are well developed. There is, however, evidence for a considerable, domestication induced, decrease of the importance of olfaction in the albino rat as compared to wild form (Stephan, 1954).

The olfactory part of the cerebrum consists of the bulbus and pedunculus olfactorius and the caudally adjacent cortical region (piriforme lobe and a small part of the piriforme lobe). The fila olfactoria pass through the ethmoid bone and enter the ventral surface of the olfactory bulb, a club- shaped extension of the rostral end of the telencephalon. The entering olfactory nerves form a lamina fibrosa along its surface and connect by way of the granular cells with the processes of mitral cells in the subjacent lamina glomerulosa. The mitral cell axons form the

lamina medullaris and continue as the tractus olfactorius (Kreiner, 1934; Schulz *et al*., 1972). On the dorsal surface of the olfactory bulb a similarly constructed lentiform area, theaccessory olfactory bulb, receives the vomeronasal nerves from the vomeronasal organ.

The olfactory peduncle, which includes the medial olfactory tract and intermediate, extends from the base of the bulb to the lateral olfactory tubercule runs caudally as a white band over the olfactory tubercle to the lateral olfactory tubercle. From there its fibres radiate into the lobus prepiriformis.

The prepiriformis lobe, laterally limited by the fissure rhinalis, borders the olfactory tubercle medially. Caudally lie the superficial parts of the corpus amygdaloideum (lateral olfactory nuclei, medial nuclei,cortical nuclei), for the projection of the olfactory tract, (White, 1965).

Optic nerve (II): Both optic fascicles (Retina and fasciculus opticus) (nerves) unite intracranially at an acute angle as the optic chiasma. It is located medial to the olfactory tubercle and rostral to the tuber cinereum. After a partial exchange of fibres at the chiasma, the optic tract turns around the crus cerebri and radiates into the corpus geniculatum laterale, the pretectal region, and the rostral colliculi (Volkmann, 1926; Schimke and Ehrenbrand, 1968).

Oculomotor nerve (III): The ovoid nuclei nervi oculomotorii is located at the level of the caudal colliculus in the dorsomedial part of the rostral tegmentum. Rostrally it is attached to the nuclei Edinger- westphal which contributes parasympathetic (efferent) fibres (Zeman and Innes, 1963). The oculomotor nerve forms from several roof fibres at the medial aspect of the crus cerebri. It runs through the foramen orbitorotundum and ramifies in the orbit. The ciliary ganglion is located at the site of ramification.

Trochlear nerve (IV): The small, rounded nucleus of the trochlear nerve lies caudal to the nuclei nerve III. Its root emerges dorsally between tectum and cerebellum; it runs across the crus cerebri and together with the oculomotor nerve enter the orbit.

Trigeminal nerve (V): The trigeminal nerve is associated with 6 nuclei (Wünscher *et al*., 1965). The most caudal one, the cadual nuclei tract trigeminal spinal nerve a continuation of the substantia gelatinosa of the spinal cord. It runs cranially in the medulla oblongata as far as the

rostrally following nuclei interpolaris tractus spinalis nervi V. This is followed by the nuclei oralis tractus spinalis nervi V, and finally by the nuclei sensibilis nervi V. The latter is located in the lateral part of the midportion of the tegmentum and ends rostrally at the same level as the oblong nuclei motorius nervi trigemini which lies just medial to it.The small nuclei tractus mesencephali nervi V is located lateral to the central gray substance of the midbrain.The nervus trigeminus arises as a large sensory and a smaller motor root from the lateral margin of pons. The sensory root contains the ganglion semilunare, well demarcated caudally by a deep groove (Gregg and Dixion, 1973). A bit further distally the trigeminal divides into its main branches, the ophthalmic (V1), the maxillary (V2) and the mandibular (V3) nerve. The ophthalmic nerve and the maxillary nerve enter the orbit through the foramen orbitorotundum. The former divides into lacrimal nerve, frontalis and nasociliaris.

The lacrimal nerve supplies the infraorbital lacrimal gland and the conjunctiva; the frontal nerveruns through the orbit dorsally, give a twig to the upper lid, and enter the skin of the frontal region. The nasociliary nerve gives off the long ciliary nerve and the ethmoidal nerve runs through the ethmoid foramen and the ethmoid bone into the nasal cavity. Here it supplies parts of the mucosa. Emerging from under the rostral edge of the nasal bone it gives twigs to the dorsal and lateral region of the nose. After leaving foramen orbitorotundum the maxillary nerve runs rostrally along the fossa pterygopalatina. Within the groove it is enlarged by the ganglion pterygopalatinum. Along its course the maxillary nerve gives off several branches. Shortly after its origin a meningeal ramus goes to the dura mater. Within foramen orbitorotundum arise the zygomatic branch, which passes the eye laterally and runs across the zygomatic arch to ramify in the skin of the buccal region. A zygomaticotemporal ramus ascends along the temporal muscle to supply the skin of the temporal region. Descending through the foramen palatinum the nerve palatinus major goes to the mucosa of the hard palate. The maxillary nerve is continued through the infraorbital canal as the large infraorbital nerve It ramifies in the area of the upper and lower lip to supply mainly the tactile hairs of this region. Before it enters the infraorbital canal it gives off *rami alveolares maxillaries caudalis* (through fine openings) to the alveoli of the molar teeth. A rostral alveolaris supplies the upper incisor.

The mandibular nerve consists of sensory and motor parts. Leaving the foramen ovale, it gives a meningeal branch to the dura mater, afterward a medial pterygoid nerve to the medial

portion of the pterygoid muscle. The mandibular nerve then divides into two major branches; cranial and caudal (Greene, 1959), which afterward give rise to the messeteric, deep temporal, buccal and lateral pterygoid nerve; and tehe auriculotemporal, lingual and mandibular alveolar nerve, respectively.

The masseteric nerve runs between the coronoid and condylar process of the mandible to enter the masseter muscle. The deep temporal nerve turns dorsally into the temporal muscle. The buccalis nerve runs between the temporal and masseter muscle to the mucosa of the cheek. The lateral pterygoidnerve supplies the lateral portion of the homonymous muscle. The auriculotemporalis nerve runs deep to the condyloid process as far as the mandibular joint, crosses under the Zygomatic processof the temporal bone and becomes dissipated as cutaneous twigs in the temporal region. Along its course it gives a branch to the buccal nerves, an articular branch to the mandibular joint, branches to the parotid gland and the rostral auricular nerve to the external ear.

The lingual nerve runs rostrally beneath the wall of pharynx. At about the level of the mandibular foramen it is joined by the chorda tympani, crosses the mandibular and sublingual duct laterally and continues toward the tip of the tongue. In its course it supplies the lesser sublingual gland and mucosa of the oral cavirty.The mandibular alveolar nerve runs along the medial aspect of the mandible to the mandibular foramen; near its origin arises the mylohyoideus nerve, for the mylohyoideus muscle, the rostral belly of the digastricus, the transverses mandibulae muscle and the skin of the chin. Within the mandibular canal alveolar branches go to the lower molars and the incisor. After leaving the foramen mentale, the n. mentalis supplies skin of the chin and mucosa of the lower lip.

Abducens nerve (VI): The small cell cluster of the abducent nerve nuclei is found close to the midline. It extends from the level of the rostral end of facial nerve nuclei to the level of the caudal pole of the motor nucleus of the trigeminal nerve, Cranial nerve VI arises from the caudal part of the pons and runs dorsal to the lateral wings of the anterior lobe of the pituitary gland. Attached to the other nerves medially it enters the orbit by way of the foramen orbitorotundum.

Intermediofacialis nerve (VII): The motor parts of the cranial nerve VII come from the nucleus facialis, which is located in the ventrolateral tegmentum. At the level of its caudal pole begins

the nucleus tractus solitaries, which extends back to the decussation of the pyramidal tract. It lies ventrolateral to the fourth ventricle and lateral to the central canal and receives afferent fibres of the intermediofacial nerve. Cranial nerve VI arises from the lateral edge of the pons medial to cranial nerve VIII. It runs through the facial canal of the petrosal bone, turns around the tympanic cavity and leaves through foramen stylomastoideum. Within the canal, in the region of the distinct ganglion geniculi, arises the nervi petrosus major. It runs rostrally and between the petrosal and pterygoid bone, runs to the ganglion pterygopalatinum as Vidi's nerve. At the end of the facial canal the chorda tympani is given off. It crosses the middle ear cavity and joins the lingual nerve. After its exit from the foramen stylomastoideum the facial nerve gives rise to the caudal auricular nerve, for the cervicoauricular muscle, the digastrics ramus for the caudal portion of the digastricus muscle and, together with it, the stylohyoideus for the homonymous muscle. Then the nerve runs rostrally and, deep to the parotid gland, divides in to its main branches: the auriculopalpebral nerve gives rise to the rostral auricular branch and the zygomatic branch. The latter crosses under the extraorbital lacrimal gland on its way to the orbicularis oculi muscle. In a groove between masseter and temporal muscle the buccales cross under the extraorbital lacrimal gland and run rostrally. Close to their origin they receive fibres from the auriculotemporal nerve (from V_3). Together with the more ventral marginal mandibular nerve they supply the muscles of the lips and cheek and subcutaneous muscles of the area of the tactile hairs.

Vestibulocochlear nerve (VIII): There are two cochlear nerve nuclei (nuclei cochlearis dorsalis and ventralis) and four vestibular nerve nuclei (nuclei vestibularis medialis, spinalis, lateralis and rostralis). The nuclei Cochlearis dorsalis lies dorsolaterally in the medulla oblongata at the level of the nuclei nervi facialis. The three layered subdivision described in other species appears to be absent (Wünscher *et al.*, 1965); only a narrow outer zone with sparse cells can be delineated from a subjacent wider layer with abundant cells.

The nucleus cochlearis ventralis is separated from the nuclei cochlearis dorsalis by a prominent glial layer (substantia gliosa cochlearis). Its caudal pole (r c. cochlearis ventralis) is located about at the level of the middle portion of the nuclei nervi facialis. It extends further rostrally than the dorsal nucleus.The location of the nuclei vestibularis medialis can be

macroscopically determined by the eminentia vestibularis medialis on the floor of the fourth ventricle.

The nuclei vestibularis spinalis adjoins it caudally and laterally. It is in turn rostrally followed by the nuclei vestibularis lateralis. The nuclei vestibularis rostralis lies rostral to the lateral nucleus in the lateral region of the rhombencephalon beneath the floor of the ventricle.

The vestibular and cochlear nerves emerge together from the lateral edge of the pons caudal to the intermediofacial nerve; they join the vestibular and spiral ganglion, respectively.

Glossopharyngeal nerve (IX): The nervi glossopharyngeus is associasted with four nuclei. Its afferent fibres join the tractus solitarius and end in its nucleus or go to the small nuclei intercalatus, which is located between the hypoglossi nerve nuclei and nuclei dorsalis motorius nerve vagi. Efferent fibres come from the nuclei ambiguus, an elongated column of cells which extends between the level of the middle part of the nuclei nerve hypoglossi and the caudal pole of the nuclei nerve facialis. Further efferent fibres are said to come from the nuclei salivatorius caudalis, which together with the nuclei salivatorius rostralis lies between the nuclei facialis and ambiguus (Zeman and Innes, 1963; Wünscher *et al*., 1965).

The nerve glossopharyngeus arises at the sulcus lateralis ventralis of the medulla oblongata rostral to the nerve vagus; both nerves go through the foramen jugulare. Running medial to the external carotid artery, it forms together with branches from the vagus - the plexus pharyngeus. Finally, it branches in to a lingualis, which represents the gustatory nerve of the caudal third of the tongue (Guth, 1957).

Vagus nerve (X): The afferent fibres of the vagus nerve run as part of the tractus solitarius to the nuclei solitarius or to the nuclei intercalatus; other fibres terminate in the nuclei tractus spinalis nervi trigemini. The efferent constituents come from the nuclei dorsalis motorius nervi vagi, the caudal pole of which lies dorsolateral to the central canal between nuclei nervi hypoglossi and nuclei tractus solitarii; as a slender column it runs rostrally beneath the floor of the ventricle. Other efferent fibres come from the nuclei ambiguus. Between spinal nerves IX and XI, the vagus nerve passes through the foramen jugulare to the bifurcation of the common carotid artery. On this course it contains the ganglion nodosum, on the proximal pole of which arises the r. pharyngeus and the ganglion jugulare. Together with the corresponding branch from the nerve

glossopharyngeus it forms the plexus pharyngeus. At the distal end of the ganglion arises the n. laryngeus cranialis, which goes to the larynx.

The vagus nerve runs along the common carotid artery and enters the thoracic cavity, turns around the right subclavian artery, on the left crosses over the aortic arch medially. The recurrent nerve runs along the dorsolateral aspect of the trachea to the larynx, where it supplies intrinsic muscles. On the way it supplies fine twigs to the trachea and esophagus. After giving off the recurrent nerves, the vagus nerve continues to the thoracic and abdominal viscera.

Accessory nerve (XI): The nervi accessorius gets fibres from the nuclei ambiguus and the ventral column of the first 5 or 6 cervical segments. The nuclei ventralis nervi accessorii is interpreted as a continuation of the latter area into the medulla oblongata

The accessory nerve arises together with the nervi vagus and accompanies it through the foramen jugulare. Then the fibres from the medulla oblongata join the vagus nerve, while the spinal fibres make up the externus. Caudal to the processus jugularis it runs dorsally with the occipital artery. It then turns caudally and receives fibres from the second to the fourth cervical spinal nerves. It runs to the trapezius, sternomastoideus and cleidomastoideus muscles.

Hypoglossus nerve (XI): The caudal part of nuclei hypoglossal nerve lies just lateral to the central canal at the level of the rostral part of the pyramidal decusssation. Further rostrally it runs ventrolateral to the central canal and finally dips beneath the floor of the fourth ventricle. The hypoglossalnerve arises from the ventral aspect of the medulla oblongata, lateral to the pyramidal tract, and runs through the hypoglossal canal. It passes between vagus nerve and accessories, crosses the external carotid artery, and lateral to the hyoid bone, enters and supplies the intrinsic muscles of the tongue. At the level of the external carotid artery, it gives off a descendens, which together with branches from the first three cervical nerves forms the ansa hypoglossi. It supplies twigs for the long muscles of the hyoid bone.

2.21 BLOOD SUPPLY TO CENTRAL NERVOUS SYSTEM

The blood supply of the brain comes from the internal carotid artery and the vertebral artery. Close to the fusion of both vertebral arteries arises the unpaired arteriosus spinalis ventralis, which runs caudally along the midline anastomosing with segmental vessels. It gives fine lateral twigs to the pia mater and larger vessels along the ventral median fissure into the spinal cord. Here they give twigs to the gray matter, alternating to the left and right sides. A dorsal spinal artery occurs irregularly. From the sulcus dorsolateralis they supply dorsal parts of the spinal cord (Tokioka, 1973). Venous drainage from the spinal cord takes place through six longitudinal channels whose total extent has not been described. The meshes of this venous plexus are narrow on the dorsal than on ventral side (Zeman and Innes, 1963).

Blood from the brain is collected by the dura sinus systems. The sinus sagittalis dorsalis runs along the dorsal margin of the falx cerebri and drains the veins of the dorsal and rostral regions of the cerebrum. Along the free margin of the falx cerebri runs the sinus sagittalis ventralis, which drains the veins of the medial aspect of the hemispheres. After taking up the cerebri magna vein, it ascends as the sinus rectus and fuses with the sinus sagittalis dorsalis and the paired sinus transverses in the confluens sinnum. Near the origin of tentorium cerebella the sinus transverses runs laterally and opens into the internal jugular vein, which leaves the skull through the jugular foramen. At the base of the brain the rostral cebral vein and the opthalmic vein open into the paired sinus cavernosus, these anastomose with each other through a wide sinus intercavernosus. After running caudolaterally the sinus cavernosus splits into a sinus petrosus dorsalis and a sinus petrosus centralis. The former drains the caudal cerebral vein and basilar vein and fuses with the sinus transverses. The latter opens into the internal jugular vein. The veins of the rhombencephalon) open into the sinus transverses or continue caudally into the vertebral vein

2.19 THE BLOOD SUPPLY TO THE CENTRAL NERVOUS SYSTEM OF RATS

The major supply of the brain comes from the internal carotid artery and the vertebral artery close to the fusion of both vertebral arteries arises the unpaired ventral spinal artery, which runs caudally along the midline anastomosing with segmental vessels. It gives fine lateral twigs to the pia mater and larger vessels along the ventral median fissure into the spinal cord. Here

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2.20 ADULT NEUROGENESIS

Neurogenesis in the brain of adult mammals occurs throughout life, and has been clearly demonstrated at two locations under normal conditions: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus. Neurons born in the adult SVZ migrate over a great distance through the rostral migratory stream and become granule neurons and periglomerular neurons in the olfactory bulb. Neurons born in the adult SGZ migrate into the granule cell layer of the dentate gyrus and become dentate granule cells. Recent studies also showed that newborn neurons in the adult brain integrate into the existing circuitry and receive functional input. Adult neurogenesis is regulated by physiological and pathological activities at all levels, including the proliferation of adult neural stem cells (NSCs) or progenitors, differentiation and fate determination of progenitor cells, and the survival, maturation, and integration of newborn neurons. Furthermore, these cells may be required for certain forms of brain function involving the olfactory bulb and the hippocampus, which is important for some forms of learning and memory. Whether neurogenesis occurs in areas of the adult mammalian brain other than the SVZ and SGZ remains controversial (Gould, 2007; Rakic, 2002). Adult neurogenesis also occurs in a variety of non mammalian vertebrates and has been extensively studied in songbirds (Chapouton *et al*., 2007; Nottebohm, 2004). This review summarizes recent findings that elucidate different aspects of regulation of adult neurogenesis in mammals and studies that address its functional significance.

2.20.1 Adult Neural Stem Cells and Adult Neural Progenitors

Adult NSCs are cells in the adult nervous system that can self-renew and differentiate into all types of neural cells, including neurons, astrocytes, and oligodendrocytes (Gage, 2000). Neurons are the functional components of the nervous system and are responsible for information processing and transmission; astrocytes and oligodendrocytes are collectively known as glia and play supporting roles that are essential for the proper functioning of the nervous system. The stem cell properties of adult NSCs have been shown *in vitro* via neurosphere and adherent monolayer cultures but have not been demonstrated convincingly *in vivo* until recently. Therefore, the term ‘‘neural progenitor’’ has been used to loosely describe all dividing cells with some capacity for differentiation.

Two types of neural progenitors can be identified in the SGZ according to their specific morphologies and expression of unique sets of molecular markers. Type 1 hippocampal progenitors have a radial process spanning the entire granule cell layer and ramify in the inner molecular layer. These cells express nestin, glial fibrillary acidic protein (GFAP), and the Sry-related HMG box transcription factor, Sox2 (Fukuda *et al*., 2003; Garcia *et al*., 2004; Suh *et al*., 2007). Although expressing the astrocyte marker GFAP, these cells are morphologically and functionally different from mature astrocytes. Type 2 hippocampal progenitors have only short processes and do not express GFAP. Type 2 cells may arise from type 1 cells, but direct evidence delineating this lineage relationship is still lacking. A recent study showed that type 2 Sox2-positive cells can self-renew and that a single Sox2-positive cell can give rise to a neuron and an astrocyte, providing the first *in vivo* evidence of stem cell properties of hippocampal neural progenitors (Suh *et al*., 2007). This study also suggested that the relationship between type 1 and 2 cells could be reciprocal. The transcription factor Sox2 is important for maintaining the ‘‘stemness’’ not only of certain types of adult stem cells including NSCs but also of embryonic stem (ES) cells; as part of a four-factor cocktail, Sox2 contributes to inducing adult somatic cells to take on an ES cell-like fate.

The SVZ is located next to the ependyma, a thin cell layer that lines the lateral ventricles of the brain. Ependymal cells have been suggested to be the adult NSCs responsible for neurogenesis in the SVZ (Johansson *et al*., 1999). Several studies have shown, however, that ependymal cells are quiescent and do not have the properties of NSCs in vitro (Capela and Temple, 2002; Doetsch *et al*., 1999). More importantly, cells within the SVZ (and less likely the

ependyma itself) contribute to long-term neurogenesis in the olfactory bulb (Consiglio *et al*., 2004). Three types of precursor cells exist in the SVZ: type B GFAP-positive progenitors, type C transit amplifying cells and type A migrating neuroblasts. Type B GFAP-positive neural progenitors in the SVZ are less susceptible to antimitotic treatment and may be relatively quiescent (Doetsch *et al*., 1999). The identification of SVZ progenitors was mainly based on morphological analysis by electron microscopy, but type C and A cells can also be identified by bromodeoxyuridine (BrdU) and 3 H-thymidine labeling and by specific molecular markers, such as Dlx2, doublecortin (DCX) and the polysialylated neural adhesion molecule (PSA-NCAM). Interestingly, the potential of SVZ progenitor cells appears to be limited, as the fate of their progeny is determined by the positional information established during early development of the central nervous system (CNS) (Merkle *et al*., 2007).

Lineage tracing studies in adult mice have demonstrated that newborn neurons, astrocytes and sometimes oligodendrocytes can be derived from cells expressing a given molecular marker, such as Nestin, GFAP, GLAS T and Sox2 (Breunig *et al*., 2007). However, these markers are expressed in heterogeneous populations of cells and it is not clear whether cells expressing these markers are the primary progenitors. Neither is it known whether a common progenitor exists in the adult brain. Although NSCs can be isolated from many areas of the adult nervous system, adult neurogenesis has only been consistently found in the SVZ and SGZ *in vivo*. It is hypothesized that the microenvironments of the SGZ and SVZ, known as the neurogenic niche, may have specific factors that are permissive for the differentiation and integration of new neurons. In the SGZ, adult hippocampal progenitors are closely apposed to a dense layer of granule cells that includes both mature and newborn immature neurons. Within this microenvironment, there are also astrocytes, oligodendrocytes, and other types of neurons. Hippocampal astrocytes may play an important role in SGZ neurogenesis. They promote the neuronal differentiation of adult hippocampal progenitor cells and the integration of newborn neurons derived from adult hippocampal progenitors in vitro (Song *et al*., 2002). Blockade of the Wnt signaling pathway inhibits the neurogenic activity of astrocytes in vitro and SGZ neurogenesis in vivo, suggesting that hippocampal astrocytes may act through Wnt signaling (Lie *et al*., 2005). SVZ progenitors are adjacent to the ependymal cell layer of the lateral ventricles. Ependymal cells express the protein Noggin that may promote SVZ neurogenesis by antagonizing signaling of the BMPs.

This is consistent with the proastrocytic role of BMPs added to cultured NSCs and during embryonic development (Lim *et al*., 2000). Ependymal cells may also promote the self-renewal of adult NSCs in the SVZ through pigment epithelium-derived factor (Ramirez-Castillejo *et al*., 2006). In addition, dopaminergic fibers are found in close proximity to SVZ precursor cells. Dopaminergic signaling may promote SVZ proliferation in vivo through the D2-like dopamine receptors (Hoglinger *et al*., 2004). Proliferating cells and putative neural progenitors in both SGZ and SVZ are closely associated with the vasculature, indicating that factors released from the blood vessels may have a direct impact on adult neural progenitors (Alvarez-Buylla and Lim, 2004; Palmer *et al*., 2000). Indeed, infusion of vascular endothelial growth factor (VEGF) promotes cell proliferation in the SVZ and SGZ, which can be blocked by a dominant-negative VEGF receptor 2 (Cao *et al*., 2004). In addition, VEGF is required for increased neurogenesis in adult mice exposed to an enriched environment or given the opportunity of voluntary exercise, which are both known to enhance adult neurogenesis.

The anatomical and functional components within the neurogenic niche in both SGZ and SVZ remain to be determined. Any diffusible molecules produced by local cells can influence neural progenitors. Neighboring cells can also exert their influence through direct cell-cell interactions. Furthermore, neural progenitors can be indirectly influenced by neurons outside of this microenvironment that are connected to neurons within the neurogenic niche through neural circuits. Both local and distal neurons can also exert direct influences on neural progenitors through the ambient levels of neurotransmitters in the neurogenic niche, or even through synaptic contacts with neural progenitors. Therefore, adult neurogenesis is subject to complex extrinsic regulation.

2.20.2 Regulation of Adult NSCs and Neural Progenitors.

The fundamental properties of a stem cell include the capacity to self-renew and multipotentiality enabling differentiation into a number of different cell types. Due to the lack of tools to directly identify adult NSCs and neural progenitors in vivo, the self-renewal and therefore the maintenance of adult NSCs and neural progenitors have not been clearly demonstrated. Neurogenesis declines with aging in both the SVZ and SGZ. Studies with neurosphere cultures suggest that the self-renewal capacity and the number of SVZ progenitors decrease significantly in aged animals (Molofsky *et al*., 2006). It is not known whether the number of neural progenitors in the SGZ also declines with aging, but neurogenesis in aged

animals can be restored to a certain extent by voluntary exercise, suggesting that these cells still have the capability of responding to extrinsic stimuli (van Praag *et al*., 2005). Bulk of the understanding of the regulation of adult neurogenesis comes mostly from studies based on cell cycle progression and imperfect markers of progenitors. Recently, new imaging tools have been developed to monitor adult neural progenitors in live subjects, although the resolution of such an approach is compromised. Despite these limitations, studies over the past decade have identified numerous molecular pathways that participate in the regulation of adult neural progenitors.

The Influence of Neurotransmitters SGZ progenitor cells reside in a microenvironment of complex neuronal networks. Dentate granule cells, the principal neurons in the dentate gyrus, receive excitatory glutamatergic inputs mainly from the entorhinal cortex and GABAergic inputs mostly from the interneurons within the dentate gyrus. In addition, neurons within the dentate gyrus receive a variety of inputs from many areas of the brain through different neurotransmitters and neural peptides. All cells within the dentate circuitry may potentially be influenced by these molecules. Therefore, the complexity of the circuitry in the neurogenic niche must be taken into account for an accurate description of the regulation of neural progenitor cells by specific neurotransmitters.

Within the dentate gyrus, the role of the NMDA receptor (one of the receptors for the excitatory neurotransmitter glutamate) in adult neurogenesis has been extensively studied. Although cell genesis in the dentate gyrus does not require a functional NMDA receptor, global NMDA receptor-dependent activity is inversely correlated with the level of hippocampal proliferation Cell, (Jang *et al*., 2007). It remains debatable whether adult hippocampal progenitors express functional NMDA receptors. Cultured hippocampal progenitors respond to glutamate by increased neuronal differentiation. However, the essential NR1 subunit of the NMDA receptor was not detectable in proliferating cells in vivo by immunohistochemistry (Deisseroth *et al*., 2004; Nacher and McEwen, 2006). In contrast to the elusive mechanism of glutamate, the neurotransmitter GABA directly depolarizes type 2 progenitors in the adult hippocampus, which results in calcium ion influx and increased expression of the neuronal differentiation factor NeuroD, suggesting that direct GABAergic input promotes the differentiation of type 2 hippocampal progenitors (Tozuka *et al*., 2005). Consistent with this observation, calcium ion channel antagonists and agonists decreased and increased neuronal differentiation in the adult hippocampus, respectively (Deisseroth *et al*., 2004).

Neural progenitors in the SVZ and SGZ are influenced by other neurotransmitters as well as by neural peptides (Jang *et al*., 2007). It is unclear whether these neurotransmitters and neural peptides exert direct effects on the neural progenitors, given that the expression of receptors for these molecules has not been extensively examined. The mechanism by which newborn neurons are regulated by signaling through certain neurotransmitters has recently been elucidated and will be discussed later.

2.20.3 The Influence of Growth Factors and Other Extrinsic Signals

Growth factors such as epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2) are potent factors for the maintenance of adult NSCs *in vitro*. *In vivo*, both factors promote proliferation in the SVZ, but only FGF2 increases the number of newborn neurons in the olfactory bulb (Kuhn *et al*., 1997). A later study found that EGF inhibits the differentiation of type C cells into neuroblasts (Doetsch *et al*., 2002). Interestingly, re-expression of ErbB2, one of the EGF receptors, can induce radial glia morphology in GFAP-positive cells in the SVZ of young adult mice, suggesting that its ligand, EGF, is indeed present in the adult SVZ (Ghashghaei *et al*., 2007). Although infusion of FGF2 does not affect SGZ proliferation in young mice, deletion of fgfr1 in the CNS decreases SGZ neurogenesis (Jin *et al*., 2003; Zhao *et al*., 2006). Therefore, FGF2-mediated signaling may play a permissive role in SGZ proliferation. Adult neural progenitors are also regulated by a variety of other extrinsic factors. Signaling through the Sonic hedgehog pathway may regulate adult neurogenesis. The neurotrophin brain-derived neurotrophic factor (BDNF) is one of the key positive regulators of adult neurogenesis. Mice deficient in p75, one of the BDNF receptors, have a smaller olfactory bulb and decreased neurogenesis in the SVZ. The regulatory role of BDNF in hippocampal neurogenesis will be discussed in detail later. The target cells of many of the extrinsic factors are unknown.

In addition to the possible direct influence on progenitor cells, these extracellular regulators could cause changes in other cell types within the neurogenic niche and exert an indirect effect on adult neural progenitors.

2.20.4 Intracellular Mechanisms

In addition to the canonical intracellular signaling pathways downstream of the growth factors, neurotrophins, and morphogens, a variety of other intracellular mechanisms have been implicated in the regulation of adult neurogenesis. Among these, several transcription factors have been shown to play critical roles in postnatal neurogenesis. TLX, an orphan nuclear receptor, and Bmi-1 are required for the maintenance of adult forebrain NSCs. Pax6 promotes neuronal differentiation of SVZ progenitors, whereas Olig2 has an opposite effect. Adult neurogenesis is also subject to epigenetic regulation. For example, adult NSCs lacking the methyl-CpG binding protein 1 (MBD1) exhibit increased genomic instability and reduced neuronal differentiation. In addition, genes involved in cell cycle regulation, DNA repair and chromosome stability are required for the proper function of adult neural progenitors. The early processes of adult neurogenesis might also be influenced by somatic gene insertions, as the retrotransposon long interspersed nuclear element-1 (LINE-1) is expressed in adult hippocampal neural progenitors in vitro.

In summary, numerous extrinsic factors and intracellular pathways have been implicated in regulating adult NSCs and neural progenitors. Many of these studies rely on systemic administration of factors, or genetic deletion of specific genes. Therefore, one cannot rule out the possibility of indirect mechanisms or of secondary effects from defects during early development. Targeting approaches that have greater specificity are needed in the future for an accurate description of molecular mechanisms of adult neural progenitor regulation.

The migration of newborn neurons neuroblasts originating from SVZ progenitors migrate tangentially up to a distance of 5 mm in the rodent. They migrate through a path known as the rostral migratory stream to the olfactory bulb, and then turn radially toward the granule cell layer and to the periglomerular cell layer after they reach the olfactory bulb (Lledo and Saghatelyan, 2005). This migration is not guided by radial glia or existing axon fibers but through ‘‘chain migration,’’ by forming elongated cell aggregates that are ensheathed by astrocytes. Although astrocytes do not appear to play an essential role in this chain migration, they may be involved in modulating the level of GABA, which has a negative effect on the speed of neuroblast migration. The olfactory bulb and septum might provide chemoattractant and repellent signals, respectively, and the repellent signals from the septum might contain the axon guidance molecules Slit 1 and Slit 2. Interestingly, newborn cells reach the olfactory bulb more quickly in adult than in

postnatal mice, although the distance they need to travel is much longer. Migration along the rostral migratory stream and in the olfactory bulb is regulated by cell-cell and cell-extracellular matrix interactions (Lledo and Saghatelyan, 2005). Disruption of EphB2/ephrin-B2 or neuregulin/ErbB4 pathways leads to severe defects in the chain migration of SVZ neuroblasts. The NCAM protein is required for the proper organization of the rostral migratory stream. Radial migration in the olfactory bulb is partially dependent on the extracellular matrix protein tenascin-R and the glycoprotein Reelin. The role of Reelin is likely to be cell nonautonomous as grafted wild-type cells were not able to migrate into the granule cell layer of the olfactory bulb in reeler mice, which have a mutant form of Reelin. Newborn neurons in the SGZ only migrate a short distance into the granule cell layer.

A recent study suggested that the protein Disrupted-in-schizoprenia (DISC1) may be involved in newborn neuron migration in the SGZ, as cells with less DISC1 migrate further into the granule cell layer and even into the molecular layer of the dentate gyrus (Duan *et al*., 2007). The migration of newborn neurons in the dentate gyrus may also be controlled by guidance cues, as these cells only migrate to the hilus or the molecular layer under pathological conditions, such as in animal models of temporal lobe epilepsy. Signaling through the Reelin pathway may be involved in such regulation (Gong *et al*., 2007).

2.20.5 Survival, Maturation and Integration of Newborn Neurons

Newborn neurons in the dentate gyrus go through several developmental stages with distinctive physiological and morphological properties (Esposito *et al*., 2005; Ge *et al*., 2006; Overstreet Wadiche *et al*., 2005; Zhao *et al*., 2006). Similar to immature neurons in the developing brain, newborn granule cells initially become depolarized in response to GABA because of their higher intracellular concentration of chloride ions. The response to GABA switches from depolarization to hyperpolarization at 2–4 weeks after neuronal birth, which coincides with the growth of dendritic spines and the onset of glutamatergic responses. Within this time window, new neurons have lower thresholds for long-term potentiation (Ge *et al*., 2007; Schmidt-Hieber *et al*., 2004). Interestingly, new neurons already form synapses with hilar and CA3 targets at 2 weeks after birth, although the complexity of these efferent synapses increases as neurons mature. Newborn neurons in the dentate gyrus display typical features of mature granule cells at 4 weeks of age, but they continue to change both physiologically and

morphologically (Ge *et al*., 2007; Toni *et al*., 2007; Zhao *et al*., 2006). The amplitude of long-term potentiation is larger in new neurons 4-6 weeks after birth, which may be mediated by the NR2B subunit of the NMDA receptor. The density of mushroom spines continues to increase after 8 weeks. In addition, spines from 4-week-old neurons are more likely to be associated with multiple-synapse boutons than older neurons. Once they mature, newborn granule cells receive similar glutamatergic and GABAergic inputs as existing neurons in the dentate gyrus (Laplagne *et al*., 2006, 2007). Although 1- to 3-week-old neurons have lower thresholds for induction of long-term potentiation, they do not display activity-dependent expression of immediate early genes at this stage (Jessberger and Kempermann, 2003). However, the animals' experience during this time window can affect the expression of activity-dependent immediate early genes in newborn neurons when they are 6 weeks old or older (Kee *et al*., 2007; Tashiro *et al*., 2007).

The initial depolarization by GABA plays a critical role in the maturation of newborn granule cells, as knockdown of the chloride ion channel NKCC1 leads to a significant defect in neuronal maturation (Ge *et al*., 2006). The DISC1 protein controls dendritic growth and physiological maturation in newborn dentate granule cells (Duan *et al*., 2007). Physiological and pathological conditions also affect neuronal maturation. For example, voluntary exercise accelerates the formation of mushroom spines. Seizure activity promotes spine formation on immature neurons. The molecular pathways mediating these effects remain to be determined.

Newborn neurons in the olfactory bulb also go through distinct stages of development (Lledo *et al*., 2006; Petreanu and Alvarez-Buylla, 2002). These neurons develop functional GABA receptors before glutamate receptors and the formation of dendritic spines (Lledo and Saghatelyan, 2005). Odor deprivation reduces the complexity of dendritic arborization of newborn cells, but these new neurons display enhanced excitability and action potential-dependent GABA release (Saghatelyan *et al*., 2005).

Many newborn neurons die within 4 weeks after birth, and their survival is subject to regulation by diverse mechanisms. In the SGZ, the survival of 1- to 3-week-old newborn neurons is influenced by the animals' experience, such as spatial learning and exposure to an enriched environment (Kee *et al*., 2007; Tashiro *et al*., 2007). Signaling through the NMDA receptor plays a cell autonomous role in neuronal survival during the third week after birth, which coincides with the formation of dendritic spines and functional glutamatergic inputs. Furthermore, the survival of NR1-deficient newborn neurons can be rescued to a certain extent

by global inhibition of neuronal activity (Tashiro *et al*., 2006). Similar to neurogenesis in the SGZ, the survival of newborn granule neurons in the olfactory bulb depends on sensory input (Petreanu and Alvarez-Buylla, 2002). Hence, the modulation of neuronal activity in both newborn and mature neurons in the circuit likely plays an essential role in the survival of newborn neurons. In addition, cell survival may be regulated by other mechanisms; for example, BDNF enhances the survival of newborn cells in the hippocampus. In summary, newborn neurons in both SGZ and SVZ are functionally integrated into the existing circuitry. The survival and the integration of newborn neurons in SGZ are largely determined during a critical time window (1–3 weeks) when these neurons are immature and display unique physiological properties, a finding that has led to the hypothesis that immature neurons may contribute to distinct forms of learning and memory, which has been examined by both computational and experimental approaches.

2.20.6 The Regulation and Function of Subventricular Neurogenesis

SVZ neurogenesis is regulated by the olfactory experience of animals (Lledo *et al*., 2006; Lledo and Saghatelyan, 2005). Deprivation of olfactory sensory inputs hinders maturation and survival of newborn neurons in the olfactory bulb, although its effects on cell proliferation in the SVZ are unclear. More specifically, sensory experience and the activity of young neurons are critical for the survival of 14- to 28-day-old neurons. In contrast, enriched odor exposure increases the survival of newborn neurons and transiently improves odor memory, suggesting a role for SVZ neurogenesis in this memory process. Further studies revealed that it was olfactory learning, not simple expo sure to odors, that enhanced SVZ neurogenesis. More 21-day-old newborn neurons survived in the olfactory bulb of mice that learned an odor discrimination task (Alonso *et al*., 2006). Moreover, negative information might have a dominant role in this enhanced survival, which coincided with the highest activated loci driven by the non reinforced odorant (Alonso *et al*., 2006). However, odor discrimination learning had no or even an opposite effect on the survival of 30-day-old newborn neurons, which did not correlate with odor-induced activity (Mandairon *et al*., 2006). Given the difference in the age of young neurons studied, olfactory learning may distinctively modulate new born neurons depending on their maturation status. Nonetheless, olfactory learning is a key regulator of SVZ neurogenesis. The regulation of SVZ neurogenesis by olfactory experience and learning leads to the hypothesis that adult SVZ neurogenesis plays a role in olfactory learning (Lledo *et al*., 2006). This hypothesis is supported

by some correlative evidence. NCAM knockout mice, in which both postnatal and adult SVZ neurogenesis are reduced, have deficits in spontaneous odor discrimination and short-term odor memory. In addition, fine olfactory discrimination was impaired in aging mice, mice heteroygous for leukemia inhibitory factor receptor (Lifr+/), and waved-1 mutant mice (a hypermorph of Tgf a), in which SVZ neurogenesis were greatly reduced. However, it is not clear whether these defects in odor discrimination were directly caused by decreased neurogenesis in the adult olfactory bulb. Therefore, specific ablation approaches are needed to elucidate the functional significance of SVZ neurogenesis.

2.20.7 Implications of Hippocampal Neurogenesis in Learning and Memory

It is noted that because of the differential connectivity of the hippocampus along the dorsal-ventral (septo-temporal) axis, the dorsal hippocampus may have a preferential role in learning and memory, whereas the ventral hippocampus is involved in affective behaviors (Bannerman *et al*., 2004). In this section, the putative function of SGZ neurogenesis in learning and memory is discussed, which is supported by correlative evidence, ablation studies and computational modeling.

2.20.8 Correlations between Hippocampal Neurogenesis and Cognition

Many genetic and environmental factors that affect hippocampal neurogenesis cause corresponding changes in cognitive performance. Adult hippocampal neurogenesis can be influenced by the genetic background of mice at the levels of cell proliferation, differentiation, and survival (Kempermann and Gage, 2002). A correlation between hippocampal neurogenesis and learning in a spatial memory task (the Morris water maze) was observed in mice of different strains. In addition, some mutant mice with decreased SGZ neurogenesis have impaired performance on hippocampus-dependent learning tasks, although such a correlation is not detected in every case.

In addition to genetic determinants, environment has a major impact on SGZ neurogenesis (Olson *et al*., 2006). For example, voluntary running increases SGZ cell proliferation, whereas exposure to an enriched environment promotes the survival of 1- to 3-week-old immature neurons, which are the likely substrates for experience-specific modulation (Kee *et al*., 2007; Tashiro *et al*., 2007). Both voluntary exercise and environmental enrichment

improve the performance of young and aged mice in the Morris water maze. Environmental enrichment also leads to better recognition memory (Bruel-Jungerman *et al*., 2005). Studies of mice deficient in presenilin-1, in which hippocampal neurogenesis induced by environmental enrichment was attenuated, suggested a function of neurogenesis in the clearance of memory traces in the hippocampus. Running-induced neurogenesis, which is correlated with an induction of long-term potentiation in the dentate gyrus, can be modulated by the social status of animals and is likely to be mediated by molecules such as BDNF and VEGF (Olson *et al*., 2006). In contrast to physical exercise and enriched environment, aging and stress are two major negative regulators of SGZ neurogenesis (Klempin and Kempermann, 2007; Mirescu and Gould, 2006). Although the correlation between stress and cognition is controversial (Shors, 2004), aged animals display impaired learning and memory in the Morris water maze and several other tasks. Furthermore, a correlation between SGZ neurogenesis and performance in the Morris water maze was observed in individual aged animals in some but not in other studies.

Although these studies demonstrate a correlation between the level of hippocampal neurogenesis and cognition, it is possible that other factors, such as structural plasticity and neurotrophin and hormone levels, also contribute to environmentally-induced changes in hippocampus- dependent learning and memory (Olson *et al*., 2006). In fact, hippocampal neurogenesis was reported to be dispensable for the beneficial effects of enriched environment on performance in the Morris water maze (Meshi *et al*., 2006). Hence, whether hippocampal neurogenesis is a major causal factor for the changes in cognition under these conditions is yet to be determined.

2.20.9 Modulation of SGZ Neurogenesis by Learning

The regulation of neurogenesis by neural activity suggests that learning might induce the activation of newbon neurons and subsequently enhance their survival and incorporation into circuits. However, numerous investigations have revealed that the regulation of SGZ neurogenesis by learning is complex. SGZ neurogenesis is only enhanced by learning tasks that depend on the hippocampus. The survival of 7-day-old neurons in rat SGZ is increased by trace eyeblink conditioning and by learning the Morris water maze but not by hippocampus-independent tasks, such as delay eyeblink conditioning and active shock avoidance (Leuner *et. al*., 2006). In addition, the survival of newborn neurons is increased by a long-delay conditioning

task, which requires the hippocampus. Conversely, pretraining in delayed conditioning, which makes the subsequent trace conditioning independent of the hippocampus, renders trace conditioning ineffective in promoting the survival of newborn neurons (Leuner *et al*., 2006). Moreover, it is learning and not simple training that elicits the survival effects. A correlation has been found between the survival of 7-day-old neurons and learning and memory performance of the individual rats despite the variable space effect during training (Sisti *et al*., 2007). Similarly, learning appears to promote the survival of newborn neurons only in cognitively unimpaired aged rats (Drapeau *et al*., 2007).

Furthermore, learning elicits different influences on neural precursors at different developmental stages. For instance, in contrast to the 7-day-old cells, the survival of 3-day-old newborn cells is inhibited by training in the Morris water maze through increased apoptosis. Interestingly, blocking apoptosis during the late phase of Morris water maze training led to impaired performance in rats, implicating a requirement for selective integration of newborn neurons in spatial learning and memory (Dupret *et al*., 2007). Finally, different phases of learning have different impacts on SGZ neurogenesis. The early phase of training in the Morris water maze, during which rapid improvement of performance is achieved, has no effect on cell proliferation, whereas the late asymptotic phase increases cell proliferation and decreases the survival of new cells produced in the early phase (Dobrossy *et al*., 2003). This type of intricate regulation of SGZ neurogenesis by learning was also observed in the social transmission of the food preference paradigm, a natural form of associative learning dependent on the hippocampus (Olariu *et al*., 2005).

Thus the regulation of SGZ neurogenesis by hippocampus-dependent learning is complicated and can be affected by factors such as the age of the newborn neurons, the stage of learning and specific learning protocols.

2.21. Role of sub granular zone neurogenesis in learning

To investigate the function of adult hippocampal neurogenesis in learning and memory, several experimental methods have been developed to decrease or even ablate SGZ neurogenesis in adult animals. These include (1) low-dose irradiation of either whole brain or restricted brain regions (Santarelli *et al*., 2003; Snyder *et al*., 2005); (2) systemic treatment with antimitotic drugs, such as methylazoxymethanol acetate (MAM) (Shors *et al*., 2001); (3) using aging as a

natural process to reduce neurogenesis and (4) using genetically engineered mice to specifically eliminate neural progenitors, such as the GFAP-tk mice in which the proliferating GFAP+ progenitors are susceptible to ganciclovir treatment (Saxe *et al*., 2006). Although none of these methods specifically targets adult progenitors, they begin to reveal a causal link between SGZ neurogenesis and cognition and suggest several potential roles for SGZ neurogenesis.

The function of neurogenesis in learning was first examined by Shors and colleagues (Shors *et al*., 2001) in MAM-treated rats that failed to form conditioned responses in trace eyeblink conditioning or trace fear conditioning but not contextual fear conditioning, which are all hippocampus-dependent tasks. Similarly, no defect in contextural fear conditioning was detected in tlx conditional knockout mice, in which hippocampal neurogenesis was greatly reduced (Zhang *et al*., 2008). In contrast, irradiated rats or mice and ganciclovir-treated GFAP-tk mice are defective in learning contextual fear conditioning tasks (Saxe *et al*., 2006; Winocur *et al*., 2006). Besides the different cell ablation methods and conditioning protocols used in these studies, only very young cells were targeted by the MAM treatment, whereas both young cells and mature newborn neurons were affected in the irradiated animals and GFAP-tk mice. Therefore, it is possible that immature and mature newborn neurons play different roles in learning, given their distinct electrophysiological properties.

The role of hippocampal neurogenesis in spatial learning and memory is rather elusive (Leuner *et al*., 2006). X-ray irradiation of 2-month-old mice leads to impaired spatial learning and memory in the Barnes maze but not in the Morris water maze (Raber *et al*., 2004). In contrast, X-ray irradiation of 3-week-old mice leads to opposite observations: impaired spatial learning and memory in the Morris water maze but not in the Barnes maze (Rola *et al*., 2004). Furthermore, many other studies were not able to detect any defects in spatial learning in animals with reduced or abolished neurogenesis (Madsen *et al*., 2003; Meshi *et al*., 2006; Saxe *et al*., 2006; Shors *et al*., 2002; Snyder *et al*., 2005), despite the fact that impaired long-term retention of memories was observed in irradiated rats and in tlx mutant mice (Snyder *et al*., 2005; Zhang *et al*., 2008). Inducible deletion of tlx in adult mice also led to defective learning in the Morris water maze. Intriguingly, ablation of hippocampal neurogenesis even caused an improvement in hippocampus-dependent working memory in the eight-armed radial maze (Saxe *et al*., 2007), suggesting that adult-born neurons may have distinct roles in the formation of different types of memories. Place and object recognition memories have also been examined in animals with

ablated neurogenesis. Conducting the test in an enriched context, Winocur *et al*., (2006) reported that irradiated rats had defects in the delayed nonmatching-to-sample test when there was a long time interval between sample trials and test trials. However, defective object recognition memory was not detected in irradiated or MAM-treated rats in a simpler context (Bruel-Jungerman *et al*., 2005; Madsen *et al*., 2003).

Nevertheless, Madsen *et al*., (2003) observed a defect in place recognition shortly after irradiation. In addition, MAM treatment prevented the enhancement of object recognition memory by an enriched environment (Bruel-Jungerman *et al*., 2005). Although these functional studies suggest some potential roles for SGZ neurogenesis in cognition, it is difficult to reach any definitive conclusions due to the controversial results. These discrepancies are probably due to differences in animal species and strains, the detailed behavioral procedures and the different knockdown strategies. More importantly, all the available strategies rely on global treatment of animals and may cause a variety of undesired side effects. Furthermore, all current studies have employed behavioral tasks based on lesion models where the whole hippocampus is affected. Given that new neurons in the SGZ constitute only a small part of the anatomical structure of the hippocampus, impaired behavior caused by a lack of neurogenesis will be easier to detect if the behavioral tests are aimed at challenging these new neurons. Therefore, to definitively demonstrate the functions of SGZ neurogenesis, selective ablation approaches with few side effects and specific behavioral tests need to be developed in the future.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.2 ANIMALS

3.2.1 Animal procurement and transportation

All rats were caught by local hunters using trap boxes and were obtained from various markets from Abeokuta ($7^0$9'39'N$3^0$20'54E) and Ibadan ($7^0$23'47^0N$3^0$55'0^0 E) to include the Itoku market Abeokuta, Ooje market Ibadan, Bode market Ibadan, Oranyan market Ibadan all within the South-Western region of Nigeria. They were transported in metal cages to the department of Veterinary Anatomy, University of Ibadan.

3.2.2 Housing and feeding

Cages of 40 X 20 X 15 cm (length, width and height) dimensions with rumpled paper bedding were used to house the animals and had one half kept dark by means of a metal cap in order to satisfy the nocturnal behavior of the rats, and to provide them with a dark sleeping chamber or nesting area. Food and water troughs containing rat pellets (Ladoke type®) were placed fixed to the wall of the cage at one end. The other end of the cage provided ventilation and light through screen wires. The rats were stabilized with feed and water ad libitum for 24-48hours with regulation of 12hour light and dark cycles for 1-2 days before the experiment. All the rats were also age estimated according to the studies by Ajayi (1974) who used weight estimates to classify the AGR as neonates (0-70 g), juveniles (over 70 g but below 500 g) and adult (above 500 g). Neonates used in this study were all born in captivity.

3.2.3 Animal handling

The animals were treated and used according to the guidelines of the University of Ibadan guidelines for laboratory animal handling.The animals were picked by the tail using gloved hands so as to prevent scratching and restraint using inhalational anaesthesia in a transparent glass (humidity) chamber.

3.2.4 Animal grouping

Forty male rats were utilized and grouped into Neonates (n=10), Juvenile (n=15) and Adults (n=15). Age classification was according to Ajayi (1975) based on age weight taken as follows: Neonates (16-70g), Juveniles (70-500g) and Adults (over 500g).

3.2.5. Materials

- Metal cages
- Weighing balance (Fivegoat® and Toledo mettler®)
- Dissecting set
- Dissecting board
- Bone cutter
- Cotton wool
- Inhalational anaesthetic agent (Chloroform)
- Perfusion pump (longerpump®)
- 10% buffered Paraformalaldehyde
- 4% Paraformalaldehyde (PFA) in Phosphate Buffer Solution
- 0.4M Phosphate Buffer (PB)
- 30% sucrose solution
- Anti-freeze
- Normal saline
- Ruler
- Thread
- Thermometer
- Distilled water

- Heater and magnetic stirrer
- Synthetic hand gloves
- Graded alcohol (50%, 70%, 95%, and 100%)
- Xylene
- Blocks
- Ethanol
- Gel coated slides
- Glass slides and cover slips
- Stains:
    - -Heamatoxylin and Eosin (HandE)
    - -Cresyl Violet
    - -Luxol Fast Blue stain
    - -Silver impregnation
- Antibodies
    - -Anti GFAP
    - -Anti CNPase
    - -Anti IbA-1
    - -Anti DCX
    - -Anti ki-67
- Molten paraffin wax
- Diamond pencil.
- TS-view and Motic softwares
- Sony™ digital camera

- Stereomicroscope
- Freezing microtome (Hyrax®)
- Zeiss Axioshop 2 plus microscope (Germany)
- Axiovision software
- Cryostat (Hyrax S50 Zeiss, Germany)

3.3 PREPARATION OF SOLUTIONS

3.3.1 Preparation of 0.4M Phosphate Buffer

500mL of distilled water was added to 10.6g of sodium di-hydrogen phosphate, and then shaken to dissolved and when ready, 56g of di-potassium hydrogen phosphate anhydrous was added and shaken well till it dissolved then distilled water was added to make up 1 liter. The pH was adjusted to 7.2-7.4 and then stored.

3.3.2 Preparation of 0.1M Phosphate Buffer

250mL of 0.4M phosphate Buffer was added to 750mL of distilled water to make 0.1M Phosphate buffer.

3.3.3 Preparation of 4% Paraformalaldehyde (PFA)

750mL of distilled water was heated to 60^{o}C, then 40g of PFA was added to the heated distilled water and stirred until it dissolved, and then 250mL of 0.4M PB was added, then it was filtered and stored in the refrigerator.

3.3.4 Preparation of 30% sucrose solution in PBS

300 g of Sucrose crystals was added to 900 mL of 0.1M Phosphate Buffer and was shaken vigorously.

3.3.5 Preparation of Anti-freeze

300mL of ethylene glycol, 300mL of glycerol, 300mL of distil water and 100mL of $2XPO_4$ were mixed together in a bottle and shook vigorously to mix well then was placed in the fridge.

3.3.6 Preparation of Gel coated slides (0.5% Gelatine coated slides (0.5% gel, 0.1% calciumchromsulpahte))

600 mL distilled water was heated to 60°C and 3g of gelatin was added, then 0.6g calciumchromsulpahte was added and allowed to dissolved for about 10 minutes the leave to cool to room temperature.

3.4 SEDATION AND PERFUSION

Restraint was accomplished using chloroform inhalation after which they were perfused transcardially first with normal saline and then with 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS), pH 7.4. All animals were anaesthesized with chloroform and perfused transcardially first with (0.9%) saline containing heparin followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Brains were removed shortly after perfusion and weighed. The brains were post fixed in 4% paraformaldehyde, cryo-protected with 30% sucrose for 2–3 days. Sagittal sections of the right hemisphere were cut on a cryostat (Hyrax S50 Zeiss, Germany) at 40µm. All sections were collected and arranged into 20 parallel series (adult and juvenile brains) and 10 parallel series (neonate brains) for histological staining. One series from each brain was stained for Nissl substance for cytoarchitecture of the brain regions.

3.5 DISSECTION AND BRAIN HARVESTING

With the aid of a scalpel blade, the head is decapitated at the occipito-atlantal junction, is skinned and with the aid of scissors and saw, the skull bones are chipped off to expose the brain in the cranium with the olfactory bulb extending towards the nasal bone areas.

The meninges are carefully dissected away from the frontal region, with the tentorium cerebelli nipped off to free the dorsal hindbrain region. The falx cerebri is then removed in between the bulbs to prevent breakage of the olfactory bulb from the rest of the brain structure. Ventrally, the meninges and the cranial nerves are severed to free the entire brain from the cranium. Gross examination was carried out visually with the aid of a dissection microscope, scalpel and forceps.

3.6 TISSUE PREPARATION AND SECTIONING

Brains were removed shortly after perfusion and weighed. The brains were post-fixed in 4% paraformaldehyde for 2days (for paraffin embedding processes) and 5-14days (for free floating samples), cryo-protected with 30% sucrose for 2–3 days and cut either coronally or sagittally on a cryostat into 40µm sections.All sections were collected and arranged into 20 parallel series (adult and juvenile brains) and 10 parallel series for neonates. One series each from the brains was stained for Nissl, Kluver and Barrera (myelin) and H&E to allow identification of brain structures.

Sections for Golgi staining were obtained from the frontal and occipital cortices from adult sized African giant rats. Neurons were selected only for qualitative studies from layer III, at a similar soma depth across regions where they were most predominant.

Photomicrographs were obtained with a Sony™ digital camera.

3.7 HISTOLOGICAL STUDIES

3.7.1 Haematoxylin and Eosin staining

The sections were mounted unto gel coated slides and allowed to dry overnight prior to staining (for frozen sections) but stained directly for 10µm paraffin was sections. Tissues were stained in Haematoxylin for 15 minutes with excess stain been washed off, after which they were differentiated in 1% acid alcohol in 4 seconds and washed off and blued in water for 4 minutes. The tissues were then counter stained in 1% Eosin for 3 seconds and dehydrated in 50%, 70%, 95%, and 100% ethanol. They were cleared in Xylene in 3 stages with the slides remaining in the last Xylene until they were mounted using DPX which is a good transparent mountant that has a refractive index similar to that of glass. Slides were then examined under light microscope and photomicrographs were taken.

3.7.2 Cresyl Violet (Nissl staining)

The sections were mounted unto gel coated slides and allowed to dry overnight prior to staining (for frozen sections) but stained directly for 10µm paraffin was sections. The slides were initially immersed in a solution containing equal amounts of alcohol and chloroform (1:1) overnight and then dehydrated in alcohol by placing the slides in a series of alcohol baths of decreasing concentrations (100% alcohol for 5 minutes two changes, 95% alcohol for 2 minutes,70% alcohol for 2 minutes and 50% alcohol for 2 minutes. The slides were then placed in cresyl violet stain for 1minute before being transferred into distilled water for another 1 minute. Sections are then dehydrated in graded alcohol baths of increasing concentration for the same time periods as above (i.e. firstly 50% and lastly 100% alcohol). The only exception is the 70% alcohol/acetic acid bath. This step acts as the differentiation and the amount of time the slides remain in the alcohol/acetic acid depends on the intensity of the cresyl violet stain which was monitored under a stereomicroscope until optimal staining was obtained. The slides were then cover slipped after dehydration.

3.7.3 Luxol Fast Blue (Kluver and Barrera method)

The sections were mounted unto gel coated slides and allowed to dry overnight prior to staining (for frozen sections) but stained directly for 10µm paraffin was sections.They were then dewaxed to 95% alcohol (not water), stained in Luxol fast blues for 2hours at 60^0C in a seal container to avoid evaporation of liquid. They were then rinsed in 70% Alcohol before rinsing in several changes of tap water. Tissues were differentiated in saturated Lithium carbonate solution for about a minute, rinsed in two changes of tap water while checking for differentiation under the microscope. It was then stained in Cresyl violet for 5minutes, rinsed in three changes of tap water before being differentiated in cresyl fast violet differentiator for about 2minutes. The tissues were then dehydrated from 95% into xylene before mounting with DPX and were cover slipped.

3.7.4 Silver impregnation (Golgi staining)

Fixative Solution:

3% potassium bichromate ---------- 60 mL

10% formalin ---------- 20 mL

Mix well and store at room temperature.

2% Silver Nitrate Solution:

Silver nitrate ---------- 2 g

Distilled water ---------- 100 mL

Mix well and store at room temperature.

Procedure:

1. Fix tissue blocks (less than 1 cm thick) in 10% formalin for 24 hours or more. The alternatively you can also use the above fixative solution:
2. Place tissue blocks in 3% potassium bichromate for 3-7 days. Avoid light. Change fresh solution each day.
3. Transfer tissue blocks into 2% silver nitrate solution for 1-3 day at room temperature. Avoid light. (Note: before put blocks into silver nitrate solution, use filter paper to absorb access solution. Change silver nitrate solution (less than regular use) several times until brown participates do not appear).
4. Cut vibratome sections at 60 um thick into distilled water.

5. Mount sections on to Superfrost Plus slides. Air dry for 10 minutes. Dehydrate through 95% Alcohol, 100% alcohol, clear in xylene and coverslip.

3.8 IMMUNOHISTOCHEMICAL STUDIES

3.8.1 Optimization tests

Initial optimization tests were done with varying dilution factors (1:400; 1:250; 1:100 for DCX, Ki-67, GFAP, CNPase and 1:100; 1:75; 1:50 for IbA-1) of antibodies utilized in this study as there were no reference points to their use in the AGR. This was done in order to prevent excessive background staining or under staining of tissue. Optimization tests were also done to determine the more appropriate antigen retrieval agent between citrate buffer and tris base buffer. Both yielded positive results however, the citrate buffer was more preferred and utilized in future experiments.

3.8.2 Glia Fibrillary Acidic Protein (GFAP)

One in every twentieth (adult) sections of the sections (1 in 10 for neonates) were utilized and other sections used as control as needed. Sections were washed 2 times for 10 minutes in PBS and then rinsed with Tris-Base Saline in Triton (TBST) once for 5 minutes under gentle shaking at room temperature. Sections were then treated with blocking solution, 5 % normal rabbit serum (NRbS) in TBST for 30 minutes. Tissues were then transferred into primary antibody GFAP (Santa Cruz 1:250) in TBST supplemented with 2 % Bovine Serum Albumin (BSA) and 2 % NRbS overnight at 4 ºC under gentle shaking. The next day, tissues equilibrated at room temperature followed by a 3 times 10 minutes wash in TBST under gentle shaking at room temperature. Secondary antibody, biotinylated rabbit-anti-goat (Vector lab, CA, USA; 1:250) in Tris-Base Saline (TBS) supplemented with 2 % NRbS for 60 minutes at room temperature under gentle shaking was then applied. After that, sections were washed in TBS 3 times for 10 minutes each under gentle shaking at room temperature. Thirty minutes prior to the time of use, Avidin Biotin Complex (ABC) reagent in TBS (1:100, A plus B) was applied to the tissues for 40 minutes at room temperature under gentle shaking. Sections were then washed in TBS twice at 15 minutes each under gentle shaking at room temperature followed by washing in TB (pH 7.6) two times for 10 minutes each. Section were then pre-incubated in DAB solution,

0.5 mg/ml in TB (pH 7.6) by using 2 ml per vial for 30 minutes in the dark under gentle shaking at room temperature. 35 µl of 0.5 % H_2O_2 was added to each vial and mixed adequately and allowed to develop under visual guidance until strong nuclear staining appears within the rostral migratory stream. The reaction was stopped by washing sections in TB pH 7.6, 3 times for 10 minutes each under gentle shaking at room temperature. Tissues were then washed in PB, counterstained and mounted onto 0.5 % gelatinized slides and air dried overnight. They were then dehydrated in an ascending graded series of alcohols, cleared in xylene and cover slipped with Entelan®.

3.8.3 Cyclic nucleotide 2, 3 phosphodiesterase (CNPase)

Brains were removed shortly after perfusion and weighed. The brains were post-fixed in 4% paraformaldehyde for less than 7days, cryo-protected with 30% sucrose for 2–3 days. Before sectioning, the tissue was allowed to equilibrate in 30% sucrose in 0.1 M PB at 4^0C. Sagittal sections were taken in series from tissues frozen in 30% sucrose and sectioned into 40µm thick sections on a freezing microtome (Hyrax®). The immunostaining was performed at the University of Witwatersrand Johannesburg using the free floating method. One in 20 of 40µm thick sections were used and other sections used as control as needed. All sections were stained for Nissl (with cresyl violet) and Kluver and Barera method for myelin staining to determine anatomical orientation. (CNPase) was used as immunohistochemical marker for oligodendrocytes. Sections were washed 2 times for 10 minutes in PBS and then rinsed with Tris-Base Saline in Triton (TBST) once for 5 minutes under gentle shaking at room temperature. Sections were then treated with blocking solution, 5 % normal rabbit serum (NRbS) in TBST for 30 minutes. Tissue were then transferred into primary antibody CNPase (Santa Cruz 1:100) in TBST supplemented with 2 % Bovine Serum Albumin (BSA) and 2 % NRbS overnight at 4 ºC under gentle shaking.

The next day, tissues equilibrated at room temperature followed by a 3 times 10 minutes wash in TBST under gentle shaking at room temperature. Secondary antibody, biotinylated rabbit-anti-goat (Vector lab, CA, USA; 1:250) in Tris-Base Saline (TBS) supplemented with 2 % NRbS for 60 minutes at room temperature under gentle shaking was then applied. After that, sections were washed in TBS 3 times for 10 minutes each under gentle shaking at room temperature. Thirty minutes prior to the time of use, Avidin Biotin Complex (ABC) reagent in

TBS (1:100, A plus B) was applied to the tissues for 40 minutes at room temperature under gentle shaking. Sections were then washed in TBS twice at 15 minutes each under gentle shaking at room temperature followed by washing in TB (pH 7.6) two times for 10 minutes each. Section were then pre-incubated in DAB solution, 0.5 mg/ml in TB (pH 7.6) by using 2 ml per vial for 30 minutes in the dark under gentle shaking at room temperature. 35 µl of 0.5 % H_2O_2 was added to each vial and mixed adequately and allowed to develop under visual guidance until strong nuclear staining appears within the rostral migratory stream. The reaction was stopped by washing sections in TB pH 7.6, 3 times for 10 minutes each under gentle shaking at room temperature. Tissues were then washed in PB, counterstained and mounted onto 0.5 % gelatinized slides and air dried overnight. They were then dehydrated in an ascending graded series of alcohols, cleared in xylene and cover slipped with Entelan®. Oligodendrocyte cell type morphology was classified according to the pattern of (Lubetzky *et. al*., 1993; Hardy and Reynolds, 1993 and Baumann and Phan Dinh, 2001).

3.8.4 Ionizing Calcium binding adapter molecule 1 (IbA-1)

Sections were washed 2 times for 10 minutes in PBS and then rinsed with TBST once for 5 minutes under gentle shaking at room temperature. Sections were then treated with blocking solution, 5 % normal rabbit serum (NRbS) in TBST for 30 minutes. Tissues were then transferred into primary antibody IbA1 (Santa Cruz biotech, CA, USA; 1:400) in TBST supplemented with 2 % BSA and 2 % NRbS overnight at 4 ºC under gentle shaking. The next day, tissues equilibrated at room temperature followed by a 3 times 10 minutes wash in TBST under gentle shaking at room temperature. Secondary antibody, biotinylated rabbit-anti-goat (Vector lab, CA, USA; 1:250) in TBS supplemented with 2 % NRbS for 60 minutes at room temperature under gentle shaking was then applied. After that, sections were washed 3 times for 10 minutes each under gentle shaking at room temperature. Thirty minutes prior to the time of use, ABC reagent in TBS (1:100, A plus B) was applied to the tissues for 40 minutes at room tempera ture under gentle shaking. Sections were then washed in TBS twice at 15 minutes each under gentle shaking at room temperature followed by washing in TB (pH 7.6) two times for 10 minutes each. Section were then pre-incubated in DAB solution, 0.5 mg/ml in TB (pH 7.6) by using 2 ml per vial for 30 minutes in the dark under gentle shaking at room temperature. 35 µl of 0.5 % H2O2 was added to each vial and mixed very well and allowed to develop under visual guidance until strong nuclear staining appears within the rostral migratory stream. The reaction

was stopped by washing sections in TB pH 7.6, 3 times for 10 minutes each under gentle shaking at room temperature. Tissues were then washed in PB, counterstained and mounted onto 0.5 % gelatinized slides and air dried overnight. They were then dehydrated in an ascending graded series of alcohols, cleared in xylene and cover slipped with Entelan®.

3.8.5 Doublecortin (DCX) and Ki-67

Sections were washed 2 times for 10 minutes in PBS and then rinsed with tris- buffer saline triton-X (TBST) once for 5 minutes under gentle shaking at room temperature. Sections for Ki-67 staining were subjected to antigen retrieval in citrate buffer, pH 6.0, at 750W in a microwave for 10 minutes and allowed to cool down on the bench to room temperature for 20 minutes. Sections were then washed in TBST 2 times for 5 minutes under gentle shaking and transferred into blocking solution, 5 % NGS in TBST for 30 minutes. All Tissues were then transferred into primary antibody, primary rabbit NCL-Ki-67 (Santa Cruz, 1:800 in TBST supplemented with 2 % BSA and 2 % NGS); (DCX antibody, Santa Cruz biotech, CA, USA; 1:400 in TBST supplemented with 2 % BSA and 2 % NRbS in TBST. No antigen retrieval in a microwave) respectively, overnight under gentle shaking at 4º C. The following day, the tissues were allowed to equilibrate to room temperature in 30 minutes The tissues were then washed 3 times for ten minutes in TBST Secondary antibody was then applied, biotinylated goat- anti-rabbit (Vector lab, CA, USA; 1:250 in TBST supplemented with 2 % NGS for Ki-67 and supplemented with 2 % NRbS for DCX sections) for 60 minutes at room temperature. Tissues were then washed 3 times for 10 minutes in TBST. ABC reagent (Vector lab, CA, USA; 1:100, A and B) was then applied for 40 minutes at room temperature under gentle shaking. After that, sections were then washed in TBS 2 times for 15 minutes under gentle shaking at room temperature. Tissues were then washed in TB pH 7.6 two times for 10 minutes under gentle shaking at room temperature. Sections were then rinsed three times in TBS and TB buffer and reacted with diaminobenzidine (DAB Substrate Kit, Vector), 2 ml per vial, 35 µl of 0.5% hydrogen peroxide. Under visual guidance until a strong nuclear staining was observed. Reaction was then stopped by washing sections with TB (pH 7.6) three times for 10 minutes. Sections were counterstained in haematoxylin, mounted on slides, dried and coverslipped with Depex (Serva). Control sections were processed in the same way, but without the primary anti-body and no labelled cells were observed.

3.9 NEURO-MORPHOMETRIC STUDIES

3.9.1 Gross Cerebral measurements

The following gross and histomorphometric parameters were measured and defined as thus:

1. Maximum Cortex Length (mm): the maximum length from the tip of the frontal lobe to the caudal most portion of the occipital lobe of the cerebral cortex
2. Maximum Cortex Width (mm): maximum length across the most lateral portion of the parietal lobes of the cerebral cortex
3. Cortex Volume: volume of displace fluid from a measuring cylinder by the cerebral cortex dissected at the cerebral peduncle
4. Cortex A-P (mm): maximum thickness of the dissected cerebral cortex
5. Left Olfactory Bulb Length (mm): length of the left olfactory bulb from the tip of the bulb to the rhinal sulcus
6. Right Olfactory Bulb Length (mm): length of the left olfactory bulb from the tip of the bulb to the rhinal sulcus

3.9.2 Cerebellum

1. Cerebellum Weight: weight of the dissected cerebellum (grams)
2. Relative Cerebellar Weight: cerebellar weight/brain weight X100
3. Maximum Hemisphere Width: maximum length of the lobes of the cerebellum (mm)
4. Vermis Length: maximum rostro-caudal length of the cerebellar vermis (mm)
5. Antero-Posterior: maximum dorso ventral length of the dissected cerebellar cortex (mm)

3.9.3 Histo-cerebellar measurement

1. EGL Thickness: thickness of the external granula layer (µm)
2. ML Thickness: thickness of the molecular layer (µm)

3. PCL Thickness:thickness of the purkinje cell layer (µm)

4. Purkinje Diameter: maximum diameter of the purkinje cells (µm)

5. Purkinje Density: cell counts of purkinje cells in a defined region

6. GL Thickness: thickness of the granule cell layer (µm)

3.9.4 Cell counts

Cell counts were done in studies of the astrocytre (GFAP), adult neurogenis proliferative cell counts (Ki-67), Purkinje cell density (Nissl). Counts were obtained by identifying positive cells within defined regions within a defined area (100X100microns^2). Five of such areas were counted and average cell counts were utilized.

3.10 STATISTICAL ANALYSIS

SPSS 16

Graph pad-4

3.11 PHOTOMICROGRAPHY

Digital photomicrographs were captured using Zeiss Axioshop 2 plus microscope (Germany) and Axiovision software. TS-view and Motic softwares were used for astrocyte counts, Ki-67 proliferative counts and some micrographs.

CHAPTER FOUR

4.0 RESULTS

4.1 GENERAL GROSS ANATOMY

The forebrain (frontal division of the brain), contained the right and left cerebral hemispheres**.** The cerebrum was observed to be the largest part of the brain, and laid immediately caudal to the olfactory bulb, cranial to the cerebellum and dorsal to the brain stem. It accounted for about 55% of the total brain length and over 70% of the forebrain length in all the groups. The rhinal sulcus was found on the ventrolateral aspect of the cerebrum separated by the phylogenetically more recent neocortex. The cerebral cortex, as earlier stated, was generally more smooth (lissencephalic) in all the specimens rather than folded (gyrencephalic) except for the few adults brains which showed a slight degree of gyrencephally in the putative caudomedial visual cortex and the lateral sulcus (Figures 1-3).

The olfactory bulbs were relatively big, making up about 25% of the brain size in juvenile and adult age groups. The olfactory bulbs were oval ventrorostral projections, separated from the cerebrum by the rhinal sulcus and bore the falx cerebrii which was inserted between the bulbs in this species. The olfactory bulb structure was observed to lie in the cribriform fossa of the ethmoid bone. The piriform area was observed to be a conspicuously pear shaped cortical region ventral to the middle of the rhinal sulcus region, ventral to the middle of the rhinal sulcus at the temporal pole of the cerebrum. Juvenile AGR had larger olfactory bulbs than adults and the left olfactory bulb was relatively longer across age groups (Figure 4).

In neonates, the mid brain was conspicuous and larger than the cerebellum (Figure 1) however, in the juvenile and adult AGR, mid brain was short and concealed dorsally by the association of the cerebral and cerbellar hemispheres. It was found to consist of the typical layers of the tectum, tegmentum and cerebral peduncle in dorsoventral sequence. The tectum had the corpora quadrigemina with the rostral being smaller than the caudal coliculli. The tegmentum comprised the core of the midbrain while the crura cerbri were visible on the ventral surface of the brain.

The hind brain comprised of medulla oblongata, pons and cerebellum. The pons and medulla formed portions of the brain stem. The cerebellum consisted of the three typical lobes of rodents in the median portion (rostral, central and caudal lobes), three lobes on the lateral to the median portion (lunate, ansiform, and paramedian lobes), and two prominent paraflocculars lobes

that were found deep and lateral to the median and lateral portions of the cerebellum and the paraflocullar lobe. The cerebellum of the African giant rat was observed to be globular, lying caudal to the cerebrum and dorsal to the fourth ventricles in the region of the Pons and rostral portion of the medulla oblongata. The dorsal view of the cerebellum revealed a wormlike appearance. Ventrally, the cranial nerves were seen to be typical as in other rodents across age groups. The dura meninx appeared typical as a transparent colourless membrane which turned slightly whitish upon post fixation in PFA. Three modifications of the dura were observed to include: the falx cerebri which was partly inserted typically inserted into the corpus callosum of the cerebral cortex (*falx cerebri pars cerebrii*) but had a further atypical insertion between the lobes of the olfactory bulb which shall be named the *falx cerebri pars bulbus olfactorius*. This feature made the bulb easily destroyed on removal of the brain and thus, the falx cerebrii must be severed to successfully harvest the olfactory bulb along with the whole brain. The tentorium cerebelli was typical and occurred between the transverse fissure of the cerebrum and cerebellum separating the two cortices. The diaphragm sellae extended towards the sphenoid bone and covered in part the hypophysis in the sella turcica which was largely buried and typical excised from the rest of the brain structure during brain harvesting (Figures 2 and 4).

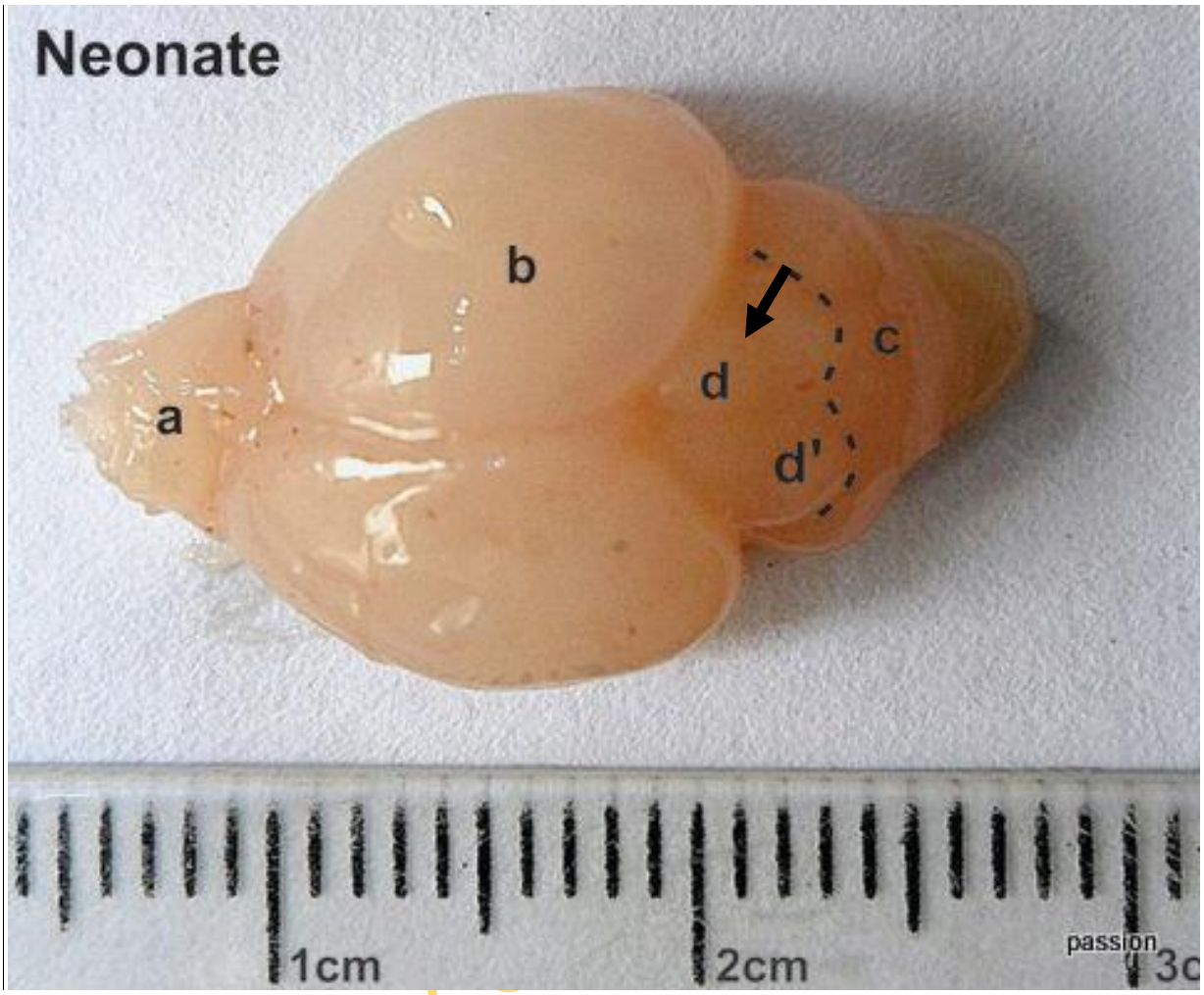


Figure 1: Photograph of neonate AGR brain showing the dorsal view. Note the separation of the cortices by the mesencephalic tectum delieanated at the caudal extent by broken dotted lines. a=olfactory bulb, b=cerebral cortex, c=cerebellum, d=rostral colliculus, d'=caudal colliculus

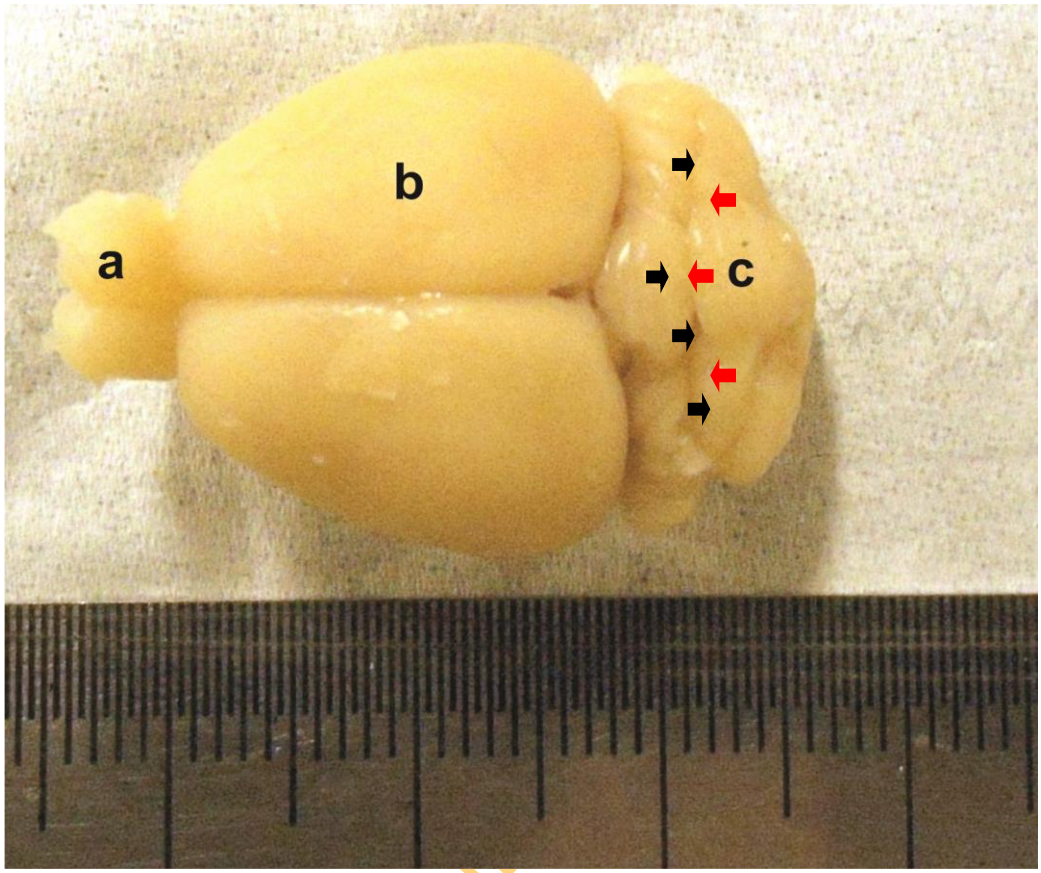


Figure 2a: Picture showing the dorsal view of a juvenile AGR brain with lissencephalic conformity and apposed cerebral and cerebellar cortices (black and red arrows). A = olfactory bulb, b=cerebral cortex, c=cerebellum

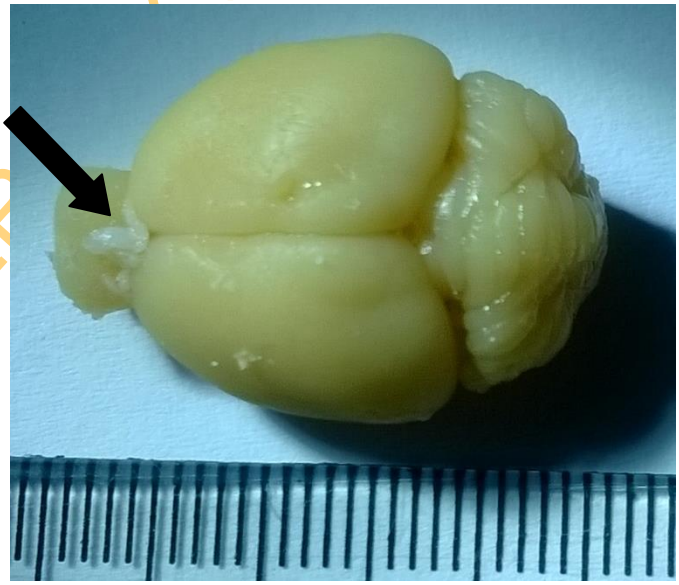


Figure 2b: Picture showing the dorsal view of a juvenile AGR brain with focus on the olfactory dura left intact for demonstration (black arrow)

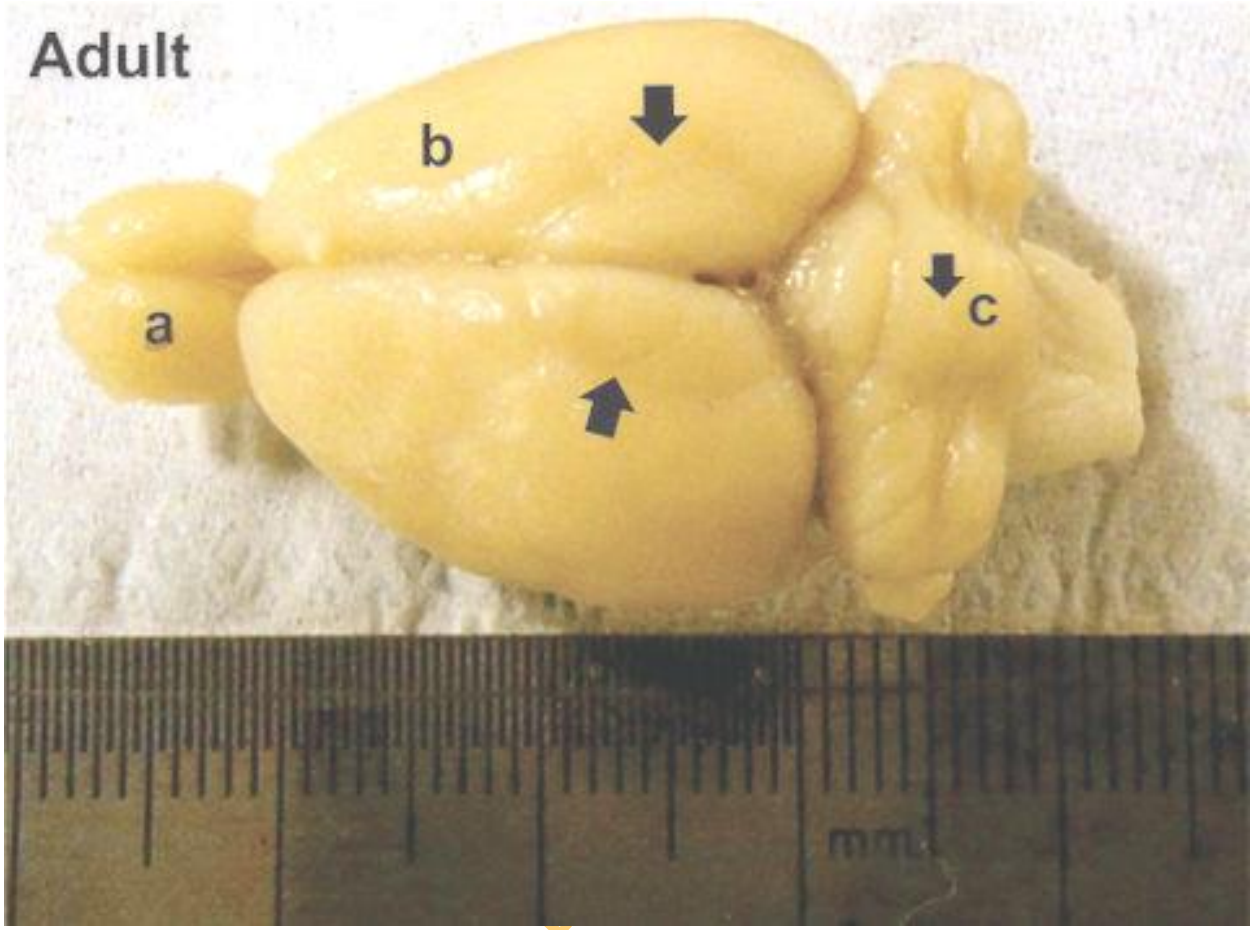


Figure 3: Picture showing the dorsal view of an adult AGR brain. The putative caudomedial visual cortex shows slight gyrencephally (arrow).a=olfactory bulb,bu cerebral cortex, c=cerebellum (note the cerebellar vermis arrow-c).

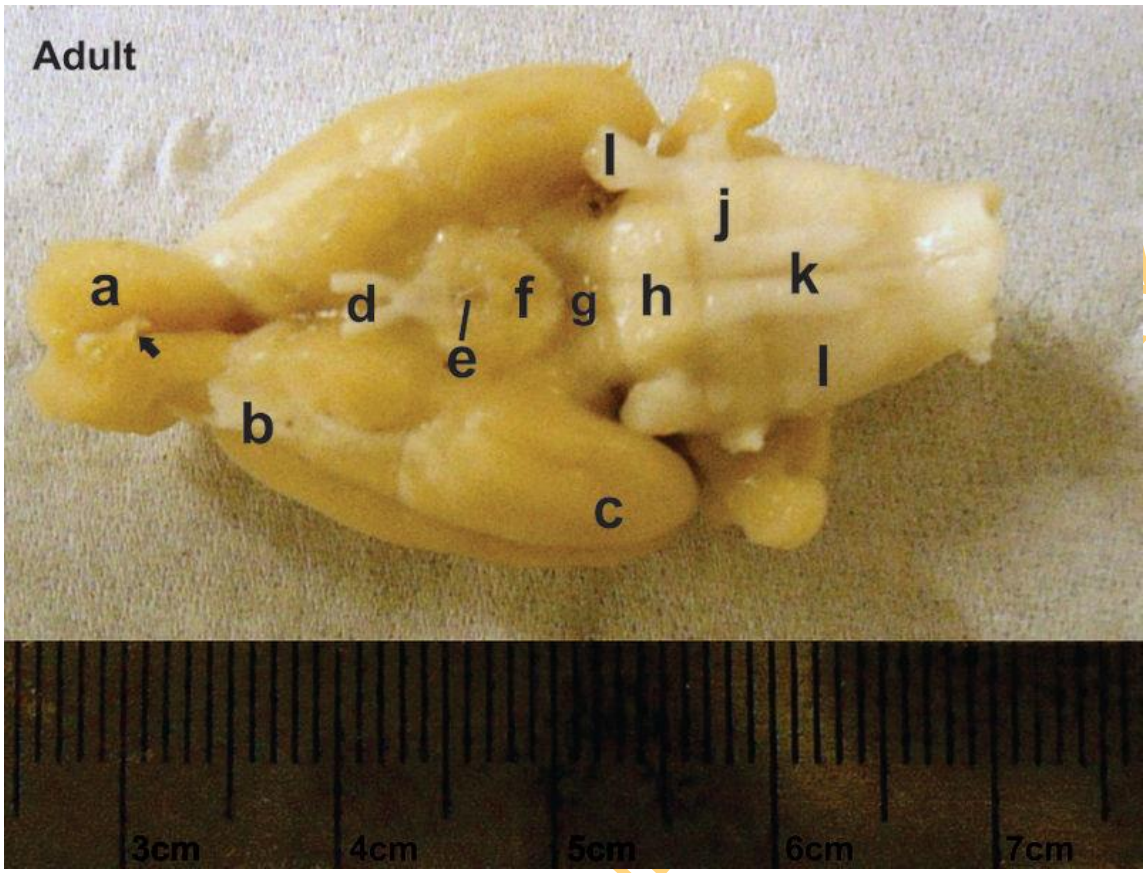
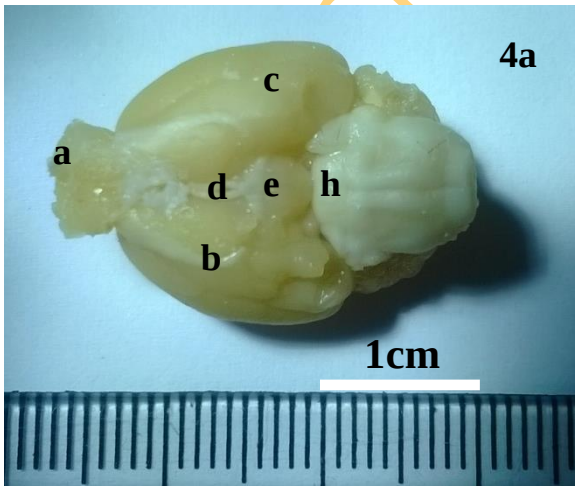
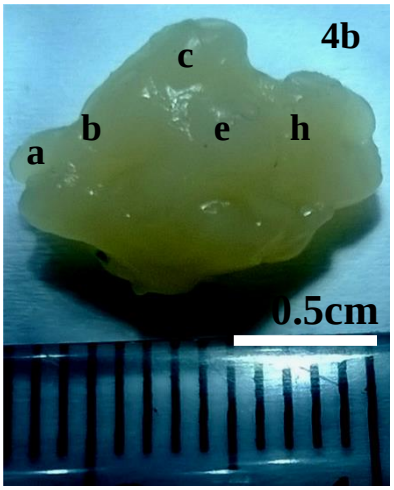


Figure 4: Picture showing the full ventral view of an adult AGR brain. a=olfactory bulb, b=olfactory tract, c=priform lobe, d=optic nerve, e=stalk of hypophysis cerebri, f=mamillary body, g=interpenduncular fossa h=pons, i=trigeminal nerve (cranial nerve V), j=trapezoid body k=pyramid, l= medulla oblongata. Note the insertion of falx cerebri par bulbous olfactorius (arrow) . Below are juvenile (4b) and neonate (4c) ventral views for comparison.





4.1.1 Mean Brain and Body Parameters

The brain weights were 5.60±0.06gm, 4.64±0.17gm and 0.62±0.08gm while the body weights were 1010.00±25.10gm, 343.11±17.78 gm and 21.65±2.40 gm for adults, juvenile and neonates respectively. The relative brain to body weight index was found to be 0.56±0.01, 1.36±0.06 and 2.85±0.16, being significantly highest in neonates and lowest in the adult group. Other body parameter such as the Tail length were 35.83±0.24, 26.00±0.00 and 3.45±0.19cm, Head length 6.83±0.18cm, 6.00±0.00cm and 2.375±0.06cm and Body length 24.67±.02cm, 18.36±0.48cm and 5.50±.44cm for adults juvenile and neonates respectively. All the parameters were statistically significant across age group with adult having the largest values followed by juveniles except the relative brain: body weight $p \leq 0.05$ (Table 4.1)

Table 4.1 The means of body parameters (given as Mean ± SEM) of adult, juvenile and neonate African giant rats.

Parameters	Stage	N	Mean ± Stderr
Brain weight (grams)	Adult	5	5.60±0.06[a]
	Juvenile	5	4.64±0.17[b]
	Neonate	5	0.62±0.08[c]
Body weight (grams)	Adult	5	1010.00±25.10[a]
	Juvenile	5	343.11±17.78[b]
	Neonate	5	21.65±2.40[c]
Relative brain weight (%)	Adult	5	0.56±0.01[c]
	Juvenile	5	1.36±0.06[b]
	Neonate	5	2.85±0.16[a]
Tail length (cm)	Adult	5	35.83±0.24[a]
	Juvenile	5	26.00±0.00[b]
	Neonate	5	3.45±0.19[c]
Head length (cm)	Adult	5	6.83±0.18[a]
	Juvenile	5	6.00±0.00[b]
	Neonate	5	2.375±0.06[c]
Body length (cm)	Adult	5	24.67±.018[a]
	Juvenile	5	18.36±0.48[b]
	Neonate	5	5.50±.440[c]

Means with same superscript letters are not significantly different at $p<0.05$. Adult AGR significantly had higher brain weight, body weight, tail length, head length and body length than the juvenile and neonates. However, neonates significantly had higher relative brain weight than juvenile and adults ($p\leq.0.05$).

4.1.2 Mean Cerebellar Parameters

With a body weight of 820.00±62.29gm, 521.25±10.77gm and 21.65±2.40gm for adults, juveniles and neonates respectively, absolute brain weights was found to be 6.08±0.22gm, 5.02±0.03gm and 0.61±0.10gm respectively. This gave a relative brain to body weight value of 0.75±0.04, 0.97±0.01 and 2.77±0.19 respectively.

The cerebellum weights were 0.83±0.02gm, 0.76±0.02gm and 0.04±0.02gm while the relative cerebellar weight values were 0.14±0.01, 0.15±0.003 and 0.12±0.005. Other parameters such as the maximum hemisphere width was 17.08±0.37cm, 16.76±0.16cm and 0.75±0.04cm ; vermis length 13.23±0.32cm, 11.27±0.014cm and 0.24±0.02cm while the antero-posterior lengths were 8.79±0.19cm, 6.97±0.03cm and 0.29±0.01cm for adults, juveniles and neonates respectively.

Adults had higher body weights, brain weight, absolute brain weight, vermis length, antero-posterior and cerebellar weight than the juvenile and neonates ($p<0.05$). However, there was no significant difference between adult and juvenile in their relative cerebellum weight and maximum hemisphere width at $p\leq 0.05$.

Table 4.2 showing means and standard error of means of adult, Juvenile and Neonate stages of animal.

Parameter	Stage	N	Mean ± Stderr
Cerebellum weight	Adult	5	0.83±0.02[a]
	Juvenile	5	0.76±0.02[b]
	Neonate	5	0.04±0.02[c]
Cerebellar weight relative	Adult	5	0.14±0.01[a]
	Juvenile	5	0.15±0.003[a]
	Neonate	5	0.12±0.005[b]
Maximum hemisphere width	Adult	5	17.08±0.37[a]
	Juvenile	5	16.76±0.16[a]
	Neonate	5	0.75±0.04[b]
Vermis length	Adult	5	13.23±0.32[a]
	Juvenile	5	11.27±0.014[b]
	Neonate	5	0.24±0.02[c]
Antero-Posterior	Adult	5	8.79±0.19[a]
	Juvenile	5	6.97±0.03[b]
	Neonate	5	029±0.01[c]
Relative brain weight (%)	Adult	5	0.75±0.04[b]
	Juvenile	5	0.97±0.01[b]
	Neonate	5	2.77±0.19[a]

Values are given as Mean ± SEM. Means with same superscript letters are not significantly different at $p \leq .05$. Adult AGR significantly had higher vermis length, antero-posterior and relative brain weight than the juvenile and neonate $p \leq 0.05$. There is no significant difference between adult and juvenile in their cerebellum weight and cerebelar relative weight $p \leq 0.05$.

4.1.3 Mean Cerebral and Olfactory Bulb Parameters

The Maximum Cortex widths for adult, juveniles and neonates respectively were 19.27±0.21cm, 20.67±0.18cm and 11.75±0.58cm while Maximum cortex lengths were 17.81±0.03cm, 18.93±0.39cm and 8.13±0.81cm. The cortex volumes were 2.84±0.04cm^3, 3.16±0.10 cm^3 and 0.23±0.02 cm^3. The antero-posterior dimensions were 11.93±0.26cm, 14.54±0.22cm and 6.00±0.16cm respectively.

Juveniles had significantly higher parameters than other groups though; there was no significance between adult and juvenile in their maximum cortex length and Right olfactory bulb at $p \leq 0.05$.

Table 4.3 showing means and standard error of means of adult, Juvenile and neonate stages of animals used in the study

Parameters	Group	N	Mean ±Stderr
Maximum cortex width (mm)	Adult	5	19.27±0.21[b]
	Juvenile	5	20.67±0.18[a]
	Neonate	5	11.75±0.58[c]
Maximum cortex length (mm)	Adult	5	17.81±0.03[a]
	Juvenile	5	18.93±0.39[a]
	Neonate	5	8.13±0.81[b]
Cortex volume	Adult	5	2.84±0.04[b]
	Juvenile	5	3.16±0.10[a]
	Neonate	5	0.23±0.02[c]
Cortex A-P (mm)	Adult	5	11.93±0.26[b]
	Juvenile	5	14.54±0.22[a]
	Neonate	5	6.00±0.16[c]
LOB	Adult	5	6.43±0.24[b]
	Juvenile	5	7.85±0.57[a]
	Neonate	5	3.25±0.19[c]
ROB	Adult	5	6.49±0.33[a]
	Juvenile	5	7.51±0.57[a]
	Neonate	5	3.13±0.24[b]

Values are given as Mean ± SEM. Means with same superscript letters are not significantly different at $p \leq 0.05$. Juvenile AGR significantly had higher max cortex width, cortex volume, Cortex A-P and Left olfactory bubl than adult and neonates ($p \leq 0.05$).

4.2 NEUROCELLULAR PROFILE

Histologically, the brain of the AGR was shown to consist broadly of five typical cell types namely: the neurons, astroglia, oligodendroglia, microglia and ependymal cells. All these cell types were observed with different stains and their morphologies were described across age groups in the AGR. For neurons, Golgi method for silver staining was used, immunostaining with GFAP, CNPase and IbA-1 gave specific orientation for glia types, Nissl staining for Ependyma cells.

4.2.1 General Laminar organization of the cerebral cortex

Six layers of the cerebral cortex were identified in this study corresponding to the typical rodent architecture. They include the plexiform (molecular layer), Outer granular layer, Pyramidal cell layer, Inner granular layer, Ganglionic layer, Multiform cell layer. No particular differences were found across age groups in terms of layering and cellular constituents. The neonates had a stratified appearance due to laminar organization of cells with predominant pyramidal cells (figures 5 and 6) and granular cells. The differentiation was clearer in the juvenile and adult groups.

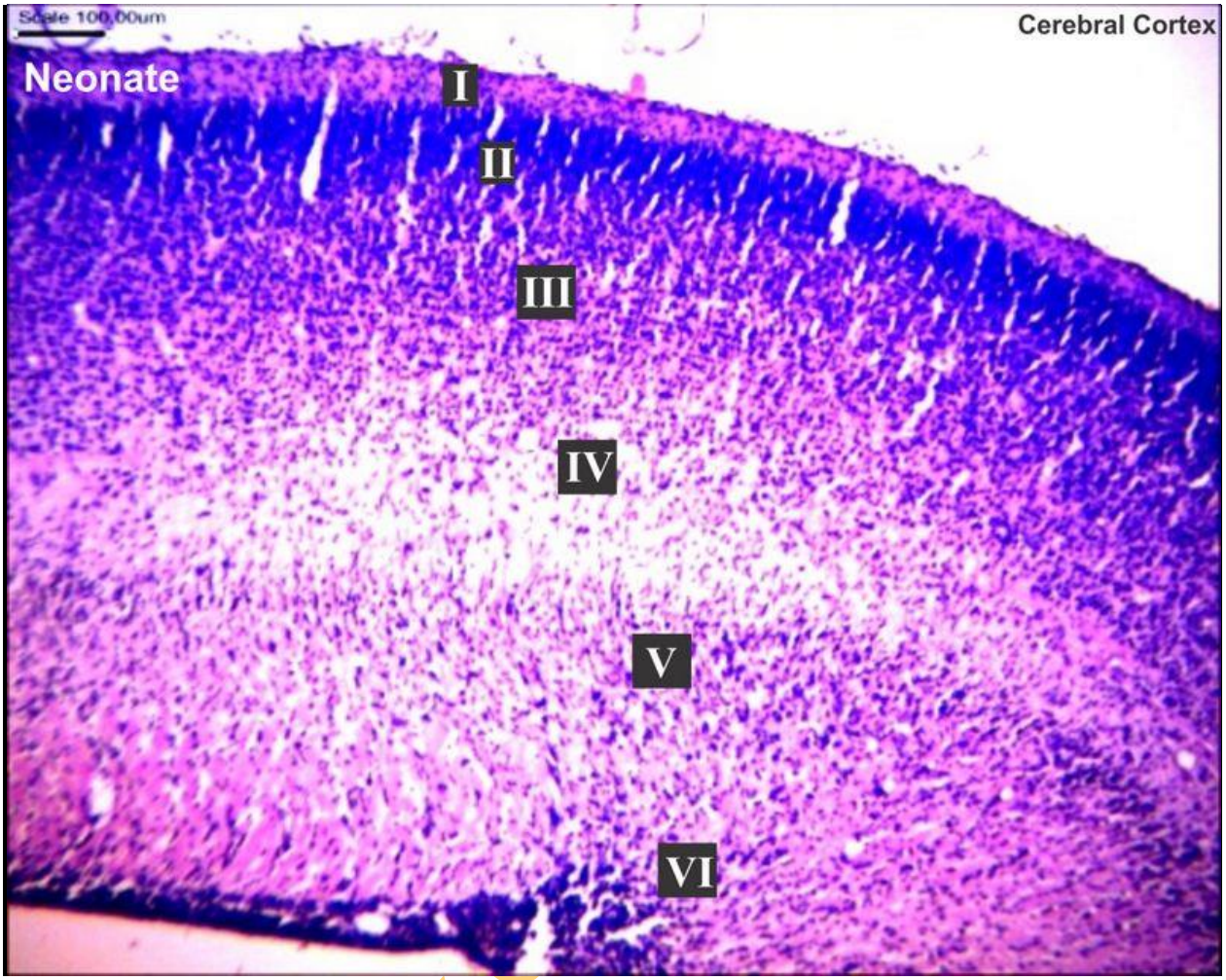
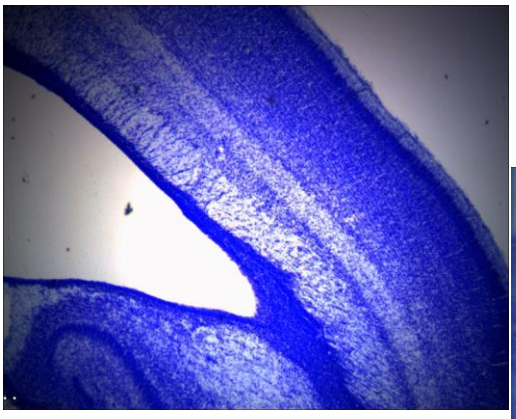


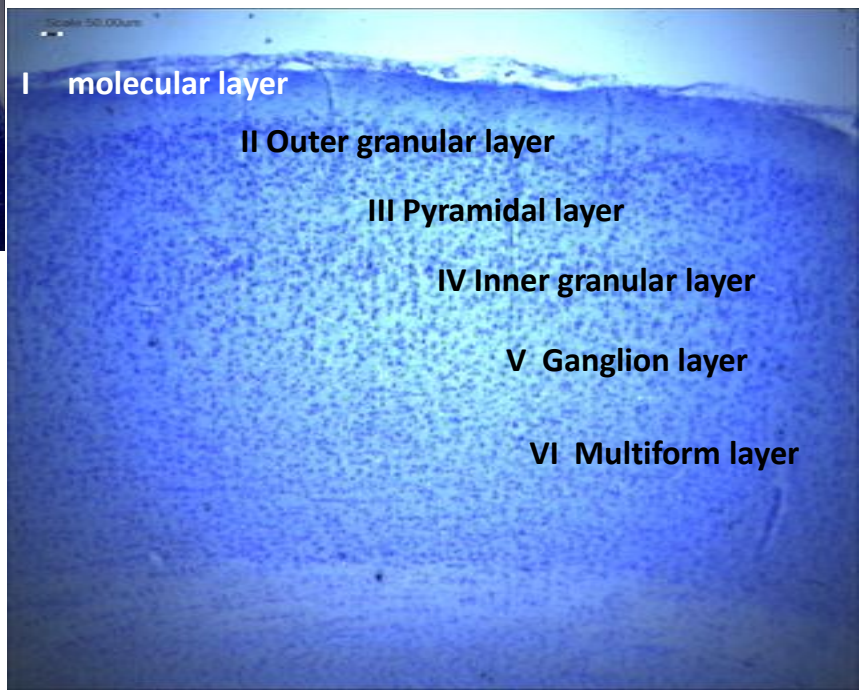
Figure 5: Representative photomicrograph showing the cerebral cortex of the neonate AGR. The various cell layers are indicated viz I= molecular layer II=outer granular layer, III=Pyramidal layer, IV=inner granular layer, V=Gang;lion cell layers VI=multiform layer H&E, scale bar-100µm



Cerebral cortex- six layers (typical)

neonates had a stratified appearance due to laminar organization of cells with predominant pyramidal cells and granule cells.

CEREBRAL CORTEX



Adult cerebral cortex, Nissl stain

Figure 6: Comparative views of the AGR neonatal (shown in insert) and adult cerebral cortex. Nissl Stain, scale bar--100µm

4.2.2 Neocortical neurons with Golgi stain

Silver staining of the cerebral cortex revealed various cell types particularly in layers II, III and V showing different neuron types including glia making synapses with neurons were identified in the brain of the AGR across age groups and they include the supragranular pyramidal neurons; multiapical pyramidal neuron; spiny multipolar cells; inverted pyramidal cells. Astrocytes, Oligodendrocytes and microglia were also identified (figures 8-12)

Supragranular pyramidal neurons: usually appeared well impregnated and typically exhibited spiny basilar dendrites extending radially from the cell body and a single apical dendrite traveling toward the pial surface. Visual inspection of relatively complete apical dendrites revealed one thick dendrite that ascended perpendicularly to the pial surface while forming thinner, but spiny, oblique branches in most cases. In some instances, however, apical dendrites originated from the soma as a single shaft, split into two thick branches that either traveled obliquely toward the pial surface, forming a V-shaped pattern, or, more frequently, ascended in parallel (Figure 7).

Glia: were observed in both cortices with synapsing observed especially between large dendritic tufts of astroglia. Oligodendroglia and microglia were rarely found but present in the preparation (figures 8-9).

Non-pyramidal multipolar neurons: were located in both frontal and occipital cortices. Their somata were either elliptic, round, or quadrangular. In all cases, dendrites emerged from different sides of the soma and spread in all directions, forming a spherical dendritic arbor. Similar morphologies characterized both the spiny and aspiny types (Fig.10a-c).

Inverted pyramidal neurons: were seen in the mid-cortical regions. These neurons exhibited a spiny basilar dendritic skirt that ascended toward the pial surface, and a thick apical dendrite with several side branches that descended toward the white matter (Fig. 10e).

Bitufted neurons: bitufted neurons were observed in layer III and V of the occipital cortex. Both spiny and aspiny types showed similar vertical morphologies, with two dendritic processes emerging from each pole of an elongated soma. A possible variant of these neurons showed an elongated soma, from which a spiny basilar skirt developed vertically in a horse tail–like fashion, giving the neuron an elliptical orientation. In contrast to bitufted neurons, these cells possessed either a single or a bifurcating apical dendrite that ascended toward the pial surface (Fig.10f).

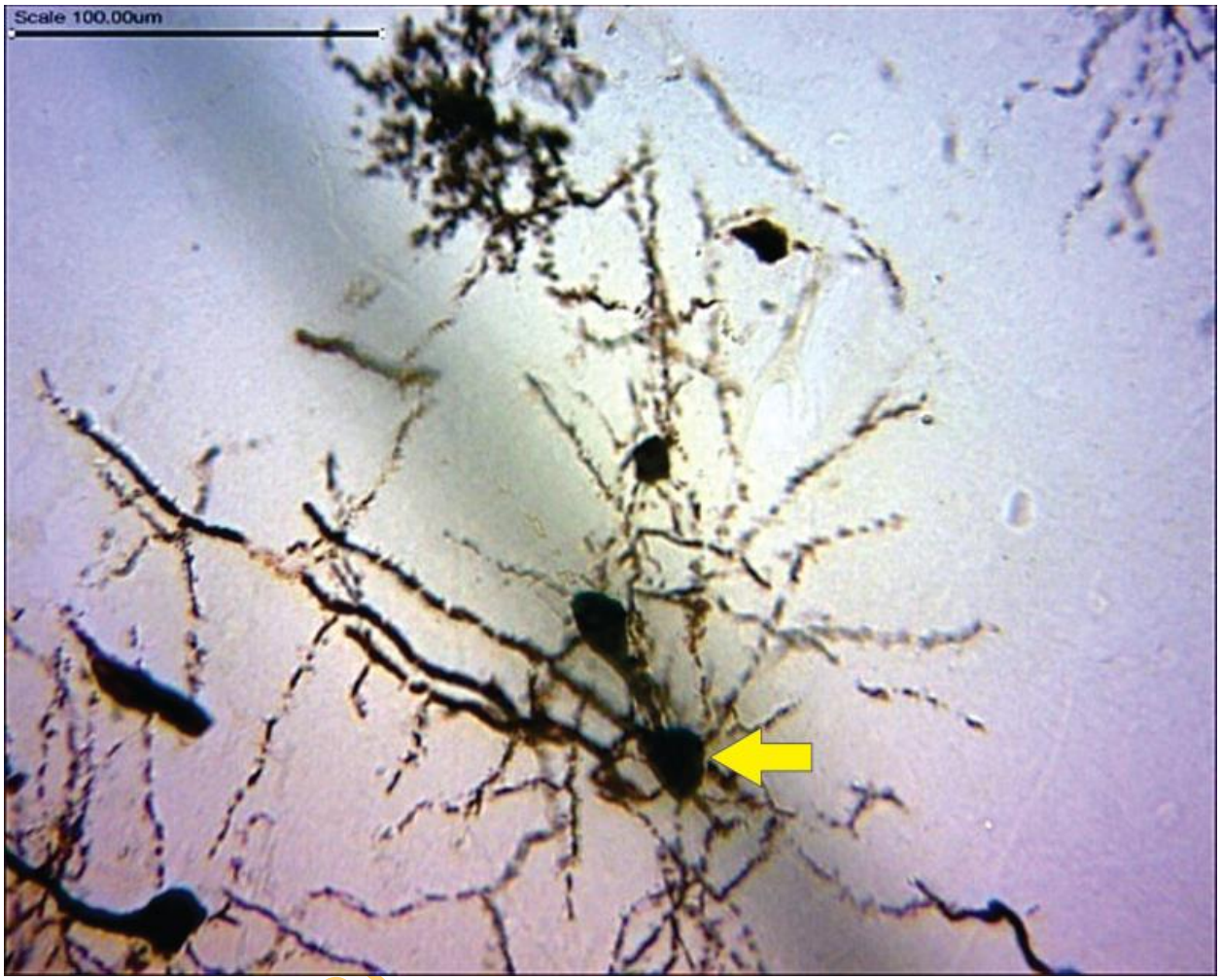


Figure 7: Representative photomicrograph of a supragranular pyramidal neuronal type in the AGR neocortex. The pial surface is towards the top of the image. Scale Bar 100µ, Golgi silver impregnation stain.

Figure 8: Representative photomicrograph showing astrocytes in synaptic association with neurons. Scale Bar: 100µm, Golgi stain.

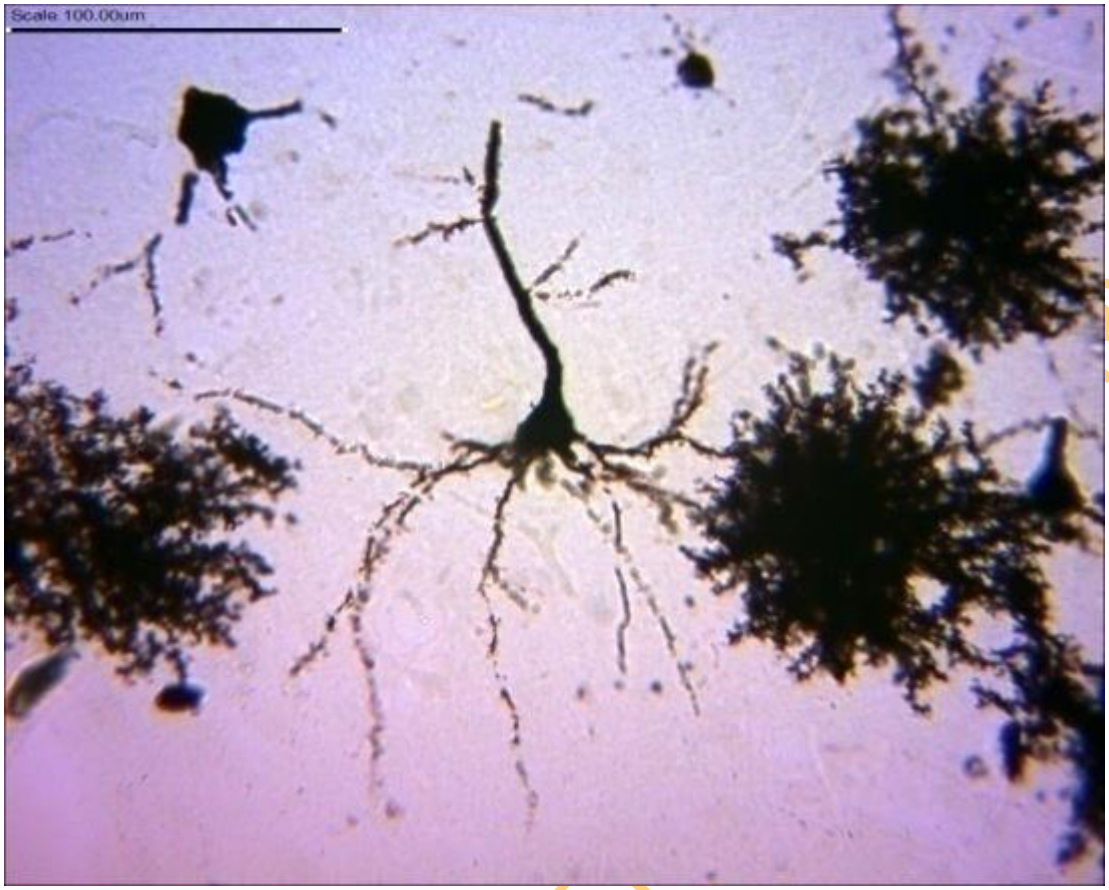


Figure 9: Representative photomicrograph showing neuronal dendrites making association with astrocytes. Scale Bar: 100µm, Golgi stain.

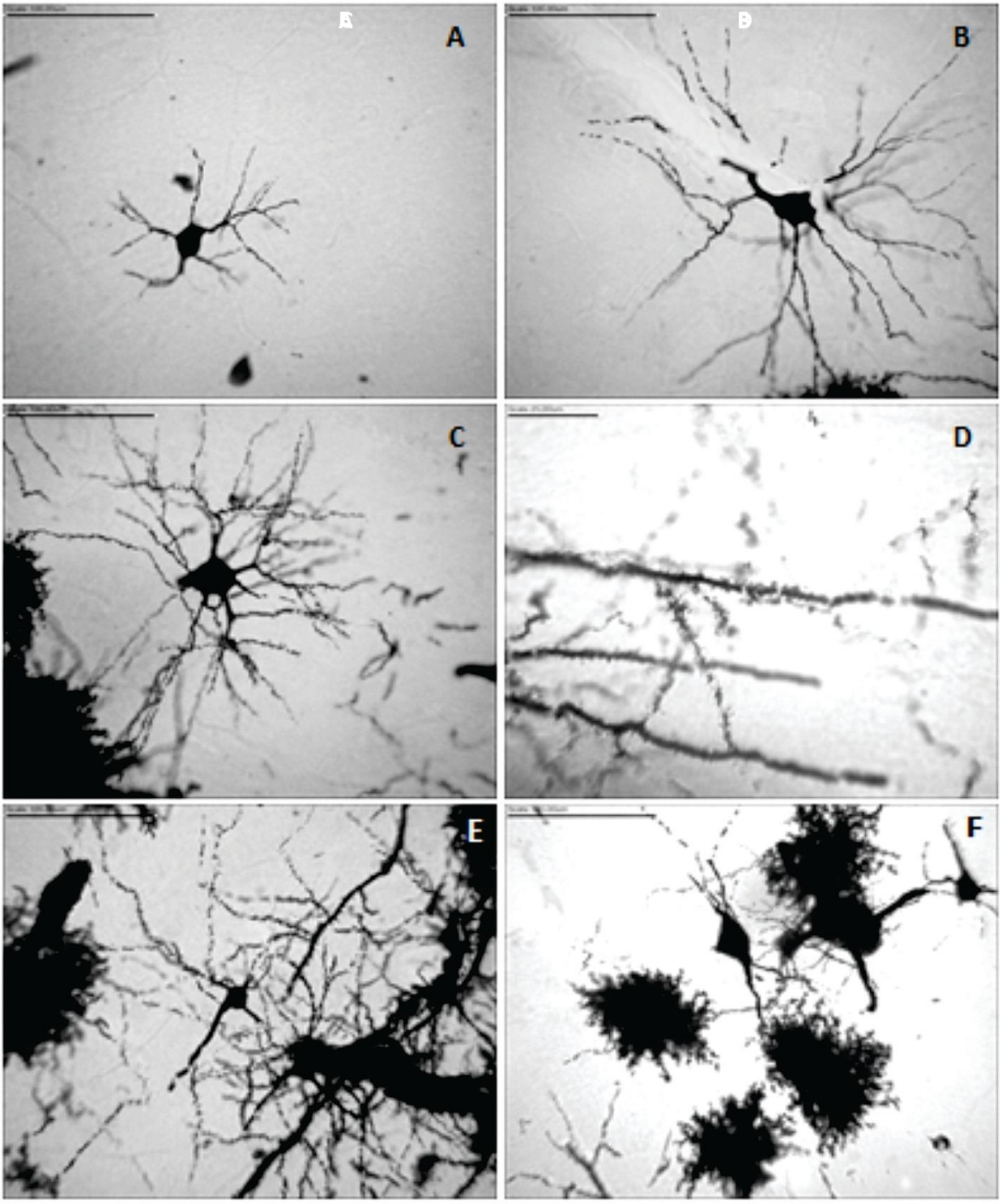


Figure 10: Representative photomicrographs showing multipolar (a-b), multiapical (c), dendritic spines (d), inverted pyramidal (e) and bipolar neurons (f). Golgi Silver impregnation stain, scale bar- 100µm

4.2.3 Ependymal Cells

The Ependymal cells formed the epithelial lining of the ventricles comprising of cuboidal or low coloumnal cells in shape, with cell appearing tightly bound together at their luminal surfaces. At the luminal surface, there was a variable number of cilia. In the lumen of the lateral ventricles in the adults and juvenile subjects, there appeared to be a stratification of the epithelium (figures 11).

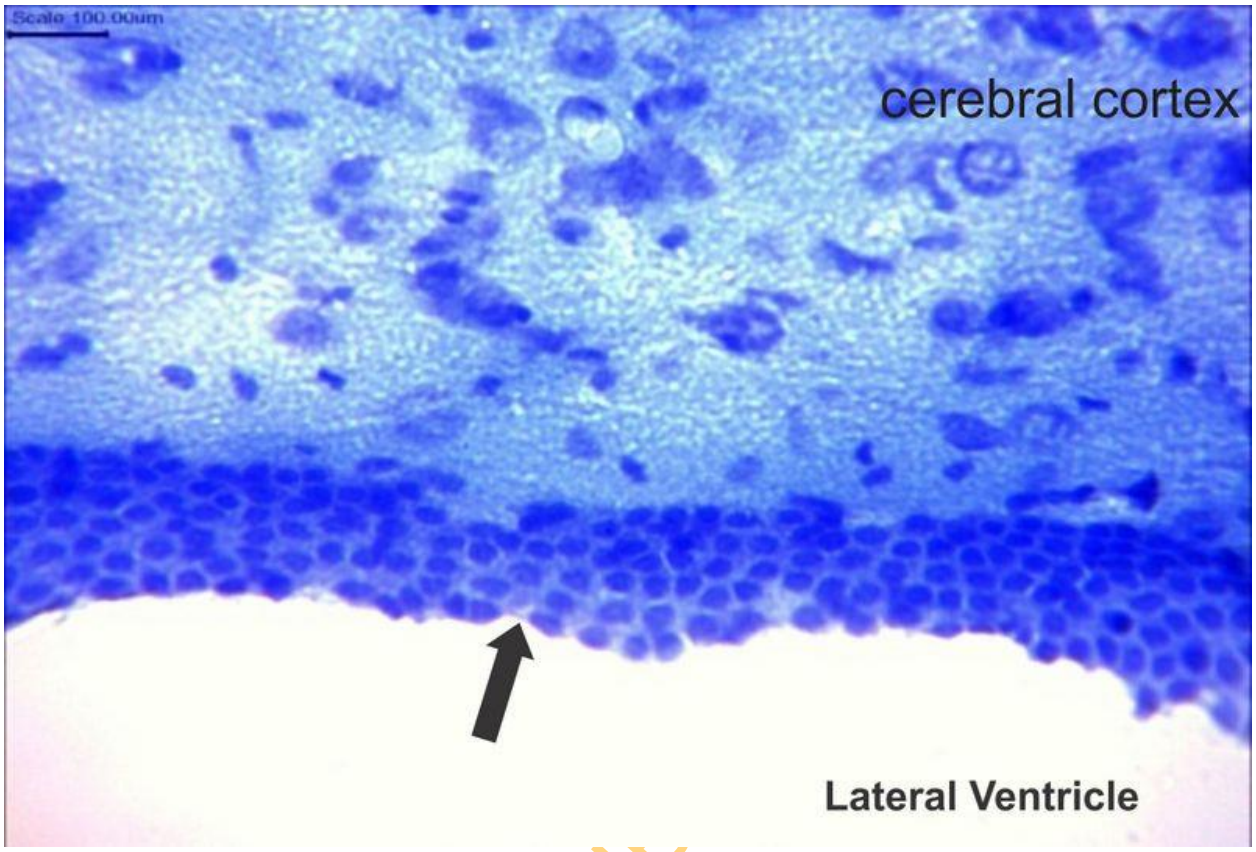


Figure 11: Representative photomicrograph showing atypical multilayered ependymocytes (arrow) a feature found across the length of the ventricular lining. Scale Bar: 100µ, Nissl stain.

4.2.4 Astrocyte studies

4.2.4.1 Immunosignalling across Age Groups

Visualizing the color intensity of GFAP immunopositive expression, juvenile sections were shown to be highest followed by adults and lastly in neonates despite using the same dilution factors for staining all sections. The regional expression intensity pattern was from caudo-rostral in all the groups. In neonates, apart from GFAP positive astrocytes being least intense, they were relatively scanty, sparse and tiny with over 90% of the observed cells detected within the glial limitans of the dorsal and ventral hindbrain, germinal zone of the fourth ventricle and mesencephalon. The expression of GFAP in the forebrain was confined to a thin line of signal extending along the hippocampal fissure and surrounding the ventricular region. Generally, low levels of signal were observed in the forebrain glial limitans and within the corpus callosum. The astrocytes appeared to have more processes than cell bodies especially in the cortical regions (Figures 12-13).

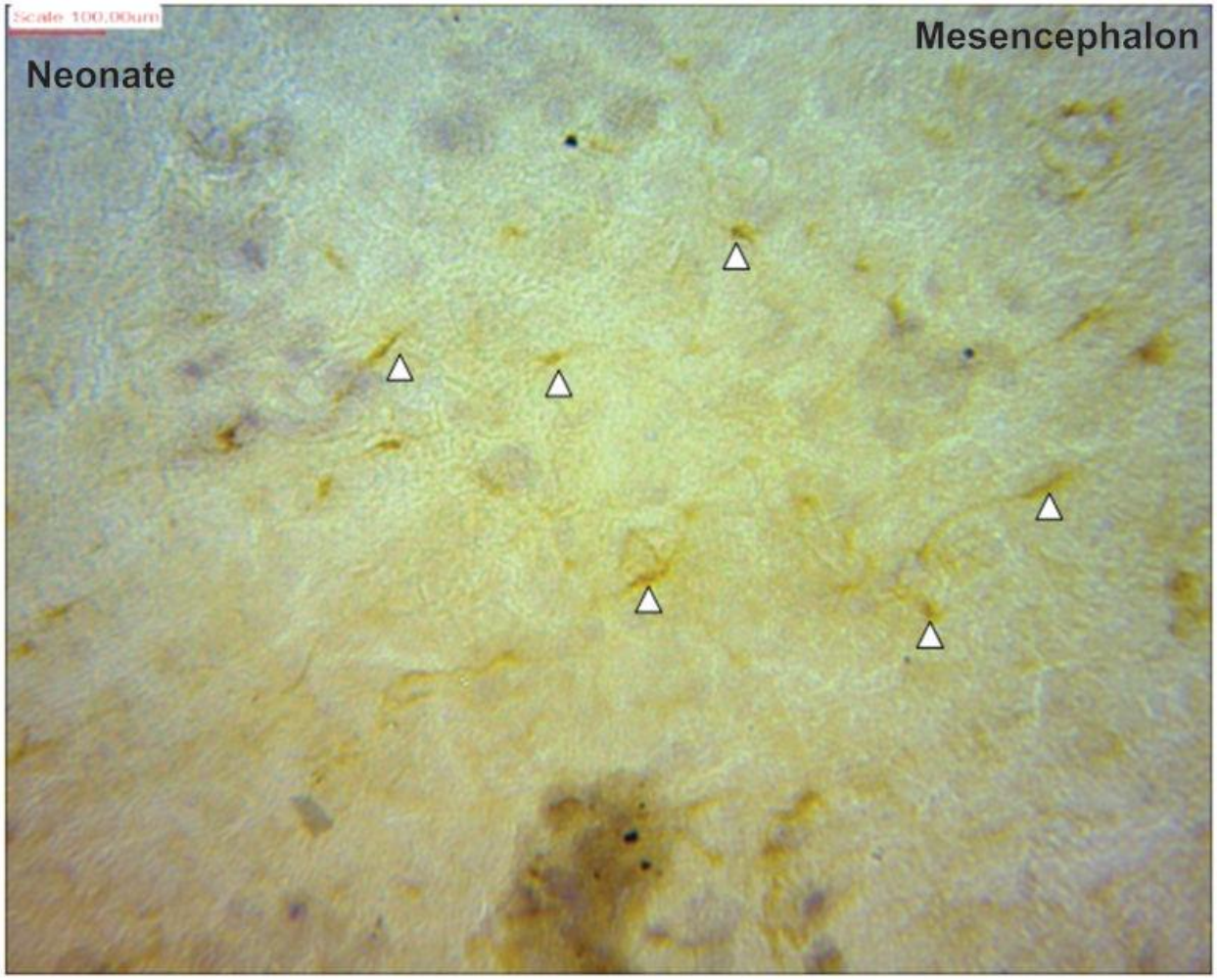


Figure 12: Representative photomicrograph showing GFAP immunopositive astrocytes in neonates with tiny processes (white arrowheads) around the mesencephallic regions. Scale Bar: 100µ, GFAP Immunostaining.

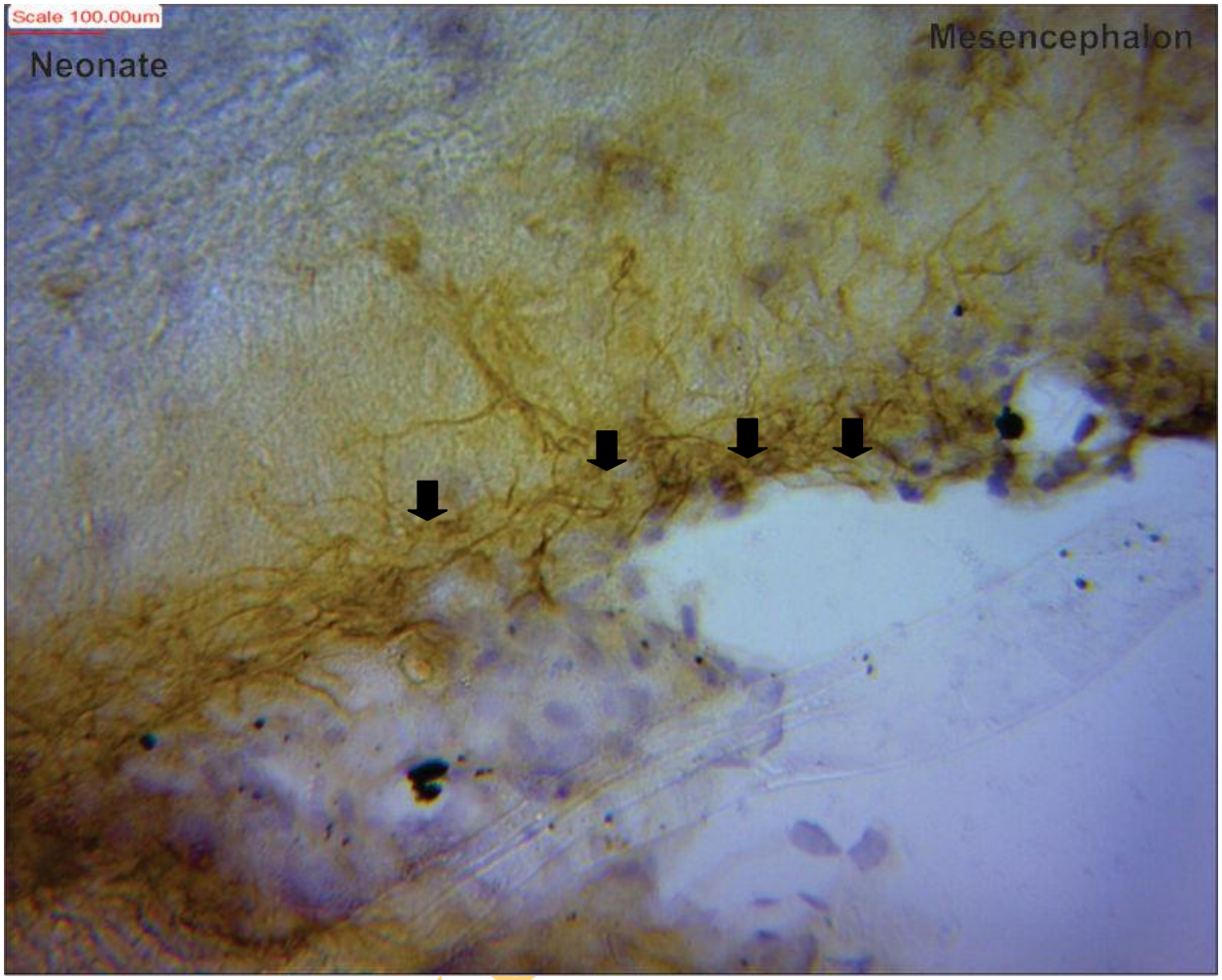


Figure 13: Representative photomicrograph showing GFAP positive immunostaining for astrocytes (arrows) in neonates at the pial surface of the mesencephalic region. Scale Bar: 100µ, GFAP Immunostaining.

It was observed that immunopositive labeling and the intensity of staining extended rostrally from mesencephalic region of neonates to the superficial regions of the cerebellum in the juvenile. This superficial labeling could be localized to the Purkinje cell and remained at moderate and reduced levels in the adult. High levels of GFAP intensity in the glial limitans of these regions had highest intensity in juvenile with reduced expression in adults. GFAP intensity in the juvenile was observed to reach apparent peak levels of expression in this group along the hippocampal area which was more prominent. This pattern decreased to more moderate levels in the adult. The appearance of strong signal within the polymorphic layer of the dentate gyrus was found in juvenile groups and was similarly reduced in the adult. Similar signal intensity was observed in the corpus callosum and hippocampal regions a feature that reduced in adult sections (Figure 14-15).

4.2.4.2 Astrocyte Morhpology

The morphology of astrocytes was seen to vary throughout the AGR brain. The location of astroglial sub-types from 12 brain sub regions were assessed and analyzed, revealing 8 astroglial subtypes in the AGR. Distinct regional heterogeneity with respect to the type of astroglial cell that resides in each neuronally defined region of the AGR brain was also observed (Table 4.4, Figure 14-15). Predominantly fibrous astrocytes were found in the corpus callosum, protoplasmic and fibrous astroglia in the hippocampus and fibrous, radial and velate astrocytes in the SVZ. From the protoplasmic astrocytes in the condensed gray matter of the olfactory bulb the cerebellar Bergmann glia and fibrous astrocytes of the brainstem, pons, cortex etc. These analyses capture considerable heterogeneity of astroglial morphologies in the AGR brain. Age group morphological differences were observed with the juvenile sections having more prominent astrocytic processes than in adults.

The astrocyte populations in the dentate gyrus of the hippocampus and corpus callosum appeared to have reactive characteristics across all juvenile and adult groups in comparison to other rodents but can be classed to be normal in this rodent since all sections observed had this morphological expression.

4.2.4.3 Astrocyte Cell Counts

Estimated cell counts were obtained using 40µm sagittal sections across the right hemisphere of the AGR brain. Estimated number of cells from 5 random mm^2 cells with an average taken. The cell population density of astrocytes were found to vary across brain regions and ranged from 1-8 cells mm^2 in the cortex to 7-17cells mm^2 in the dentate gyrus (Fig.4.5). Astroglial density also varies considerably in sub-regions of the cortex, with 1-5 in layer II-III compared to 1-8 in layer I. Age played a significant role in astrocyte population counts and morphology in different regions as there were differences in the numbers of juvenile and adults cell counts per region.

Table 4.4: Regional heterogeneity of astrocytes in the AGR brain, table shows regional heterogeneity of astrocyte subtypes found in defined brain regions in the AGR.

Region	Radial	Bergmannglia	protoplasmic	Fibrous	Velate	marginal	Perivascular
Olfactory bulb	+		+	+	+/-		
RMS				+			
Cortex			+		+/-		+
Pial surface				+		+	
Corpus callosum	+						
Lateral ventricle/SVZ	+						
Third ventricle	+						
hippocampus			+		+/-		
Dentate gyrus	+		+				
Cerebellum		+			+/-		
Brainstem				+	+/-		

Table 4.5: Astrocyte counts in different brain regions.

Brain region	Age	
	Juvenile	Adult
CORTEX*	428.33 ± 70.06	238.33 ± 36.86
PIA*	635.00 ± 185.00	163.33 ± 23.63
CORPUS CALLOSUM*	753.33 ± 83.87	358.33 ± 54.85
HIPP	580.00 ± 250.60	441.67 ± 68.98
OLFACTORY BULB	255.00 ± 80.47	204.00 ± 31.75
PERIVASCULAR	391.67 ± 143.38	300.00±141.07
RMS*	508.33 ± 30.14	261.67 ± 41.93
SUB CORTEX	206.67 ± 58.60	71.33 ± 8.08
SVZ	555.00 ± 152.23	366.67 ± 56.86
DG*	1110.00 ± 235.16	596.67 ± 80.83
CEREB. GRAN	456.67 ± 60.28	388.33 ± 46.46
CEREB.MOLE*	395.00 ± 30.41	216.67 ± 28.87
CEREB. WM	433.33 ± 51.32	343.33 ± 58.60
CEREB. PCL*	403.33 ± 25.17	306.67 ± 30.55
PONS	280.00 ± 121.24	176.67 ± 20.82
BRAIN STEM	170.00 ± 125.30	143.33 ± 30.55
3RD VENTRICLE	211.67 ± 34.03	213.33 ± 32.15

The table above shows that age influences the population of astrocytes in different regions of the brain. In all the brain regions, astrocyte count was higher in juveniles than adults except at the third ventricle. Significant difference in mean counts (*) between juveniles and adults was found in the cortex ($t = 4.16$, $p = 0.01$), pia ($t = 4.38$, $p = 0.01$), corpus callosum ($t = 6.83$, $p = 0.01$), rms ($t = 8.27$, $p = 0.01$), dg ($t = 3.58$, $p = 0.01$), cereb.mole ($t = 7.37$, $p = 0.01$) and cereb.pcl ($t = 4.23$, $p = 0.01$). The highest astrocyte count in both juvenile and adult was found in dg while the least was found in the brain stem and sub cortex respectively.

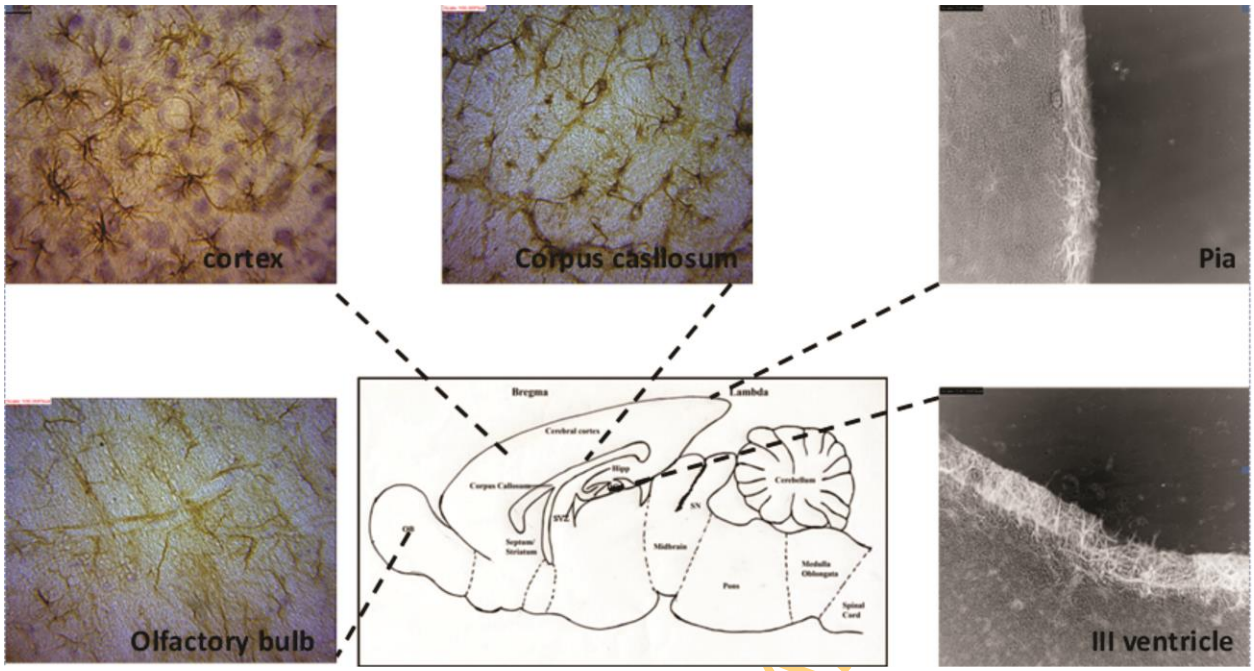


Fig 14: Representative astrocytes from selected regions of the AGR brain. Images were obtained from sagital sections. GFAP Immunostaining

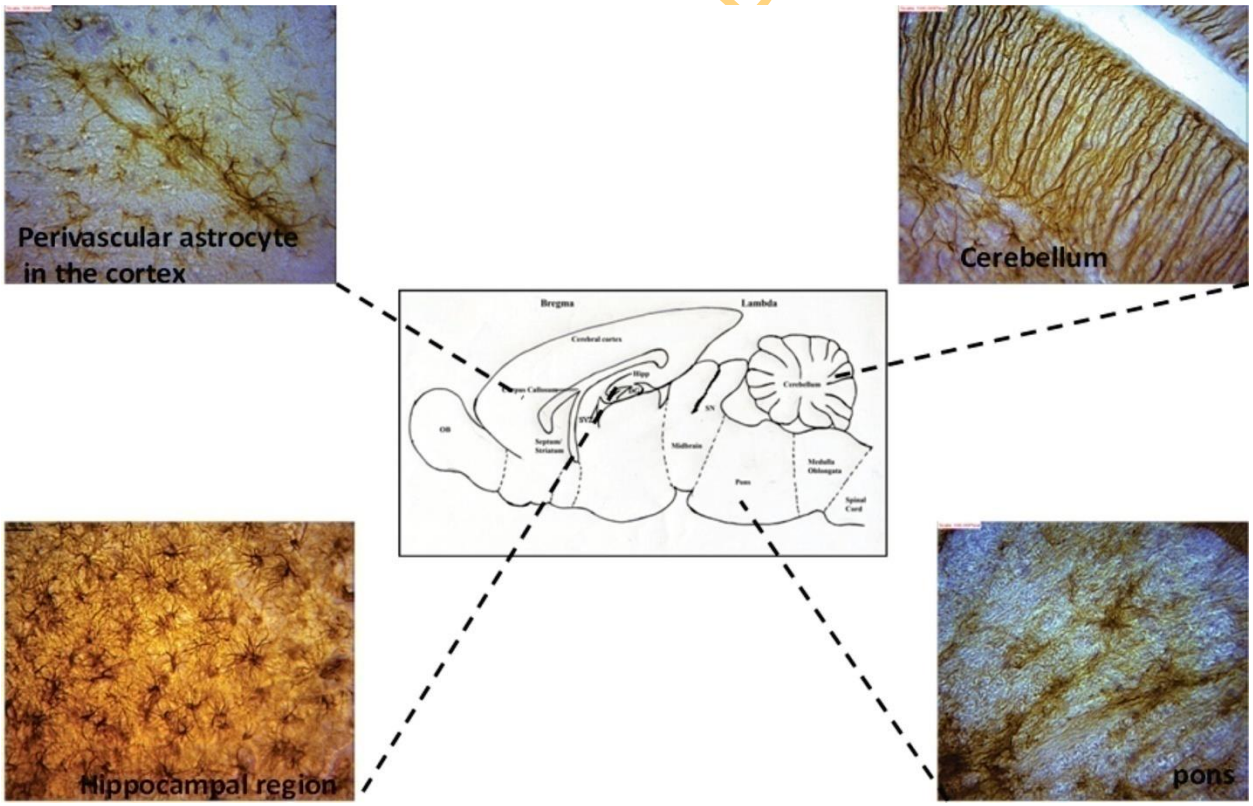


Fig 15: Representative astrocytes from selected regions of the AGR brain (images were obtained from sagittal section), GFAP Immunostaining

4.2.5 Oligodendrocyte studies

Immunostaining for CNPase was positive in all age groups delineating the dendrites and cell body in neonates plus the myelin sheath in juveniles and adults (Figure 16). Several precursor, progenitor, immature, mature non-myelinating Oligodendrocytes were observed in neonates in the cerebellar and cortical regions of the brain irrespective of the section. Juveniles and adults predominantly showed mature myelinating Oligodendrocytes with evidence of myelin sheath deposition apart from other cell types. Observed oligodendrocytes were found to include precursor, progenitor pre-oligodendrocytes, immature Oligodendrocytes, mature non-myelinating oligodendrocytes.

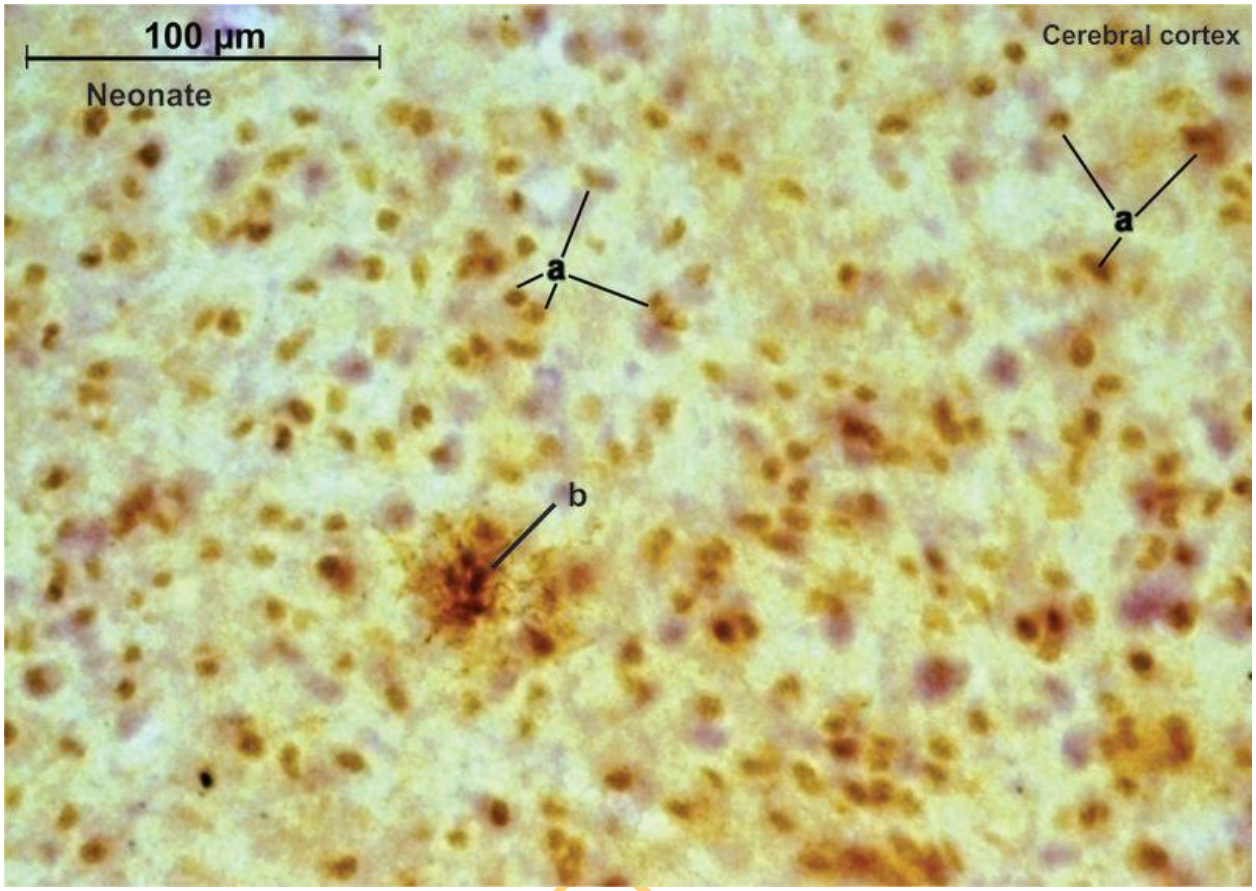


Figure 16 representative photomicrograph showing soma of precursor oligodendrocytes without dendritic extensions (a) and unmyelinating immature oligodendrocytes with dendrites (b) in neonate AGR cerebral cortex, Scale Bar 100µ, CNPase immunostaining

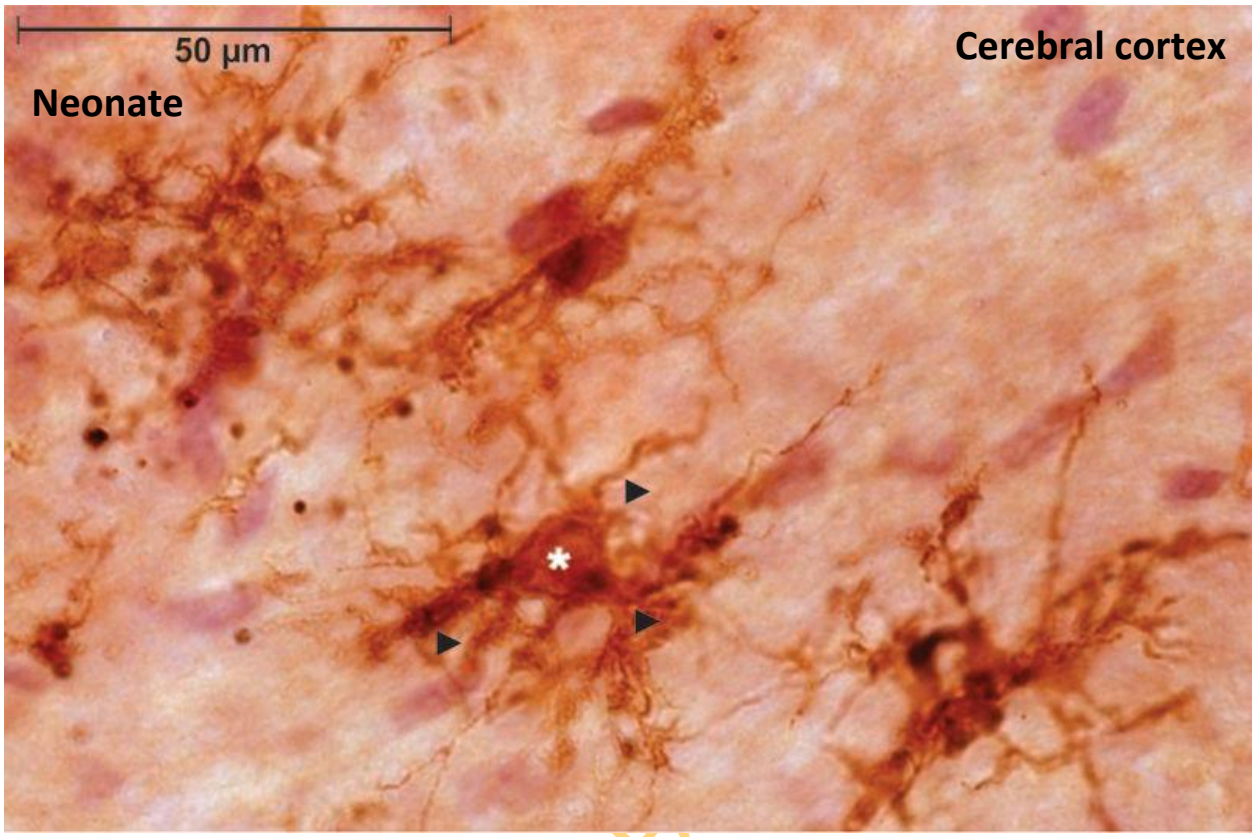


Figure 17: representative photomicrograph showing immature oligodendrocytes under oil immersion with the soma (*) and sparse dendritic extensions (arrowheads) without the evidence of myelination. Scale Bar 50µ, CNPase immunostaining

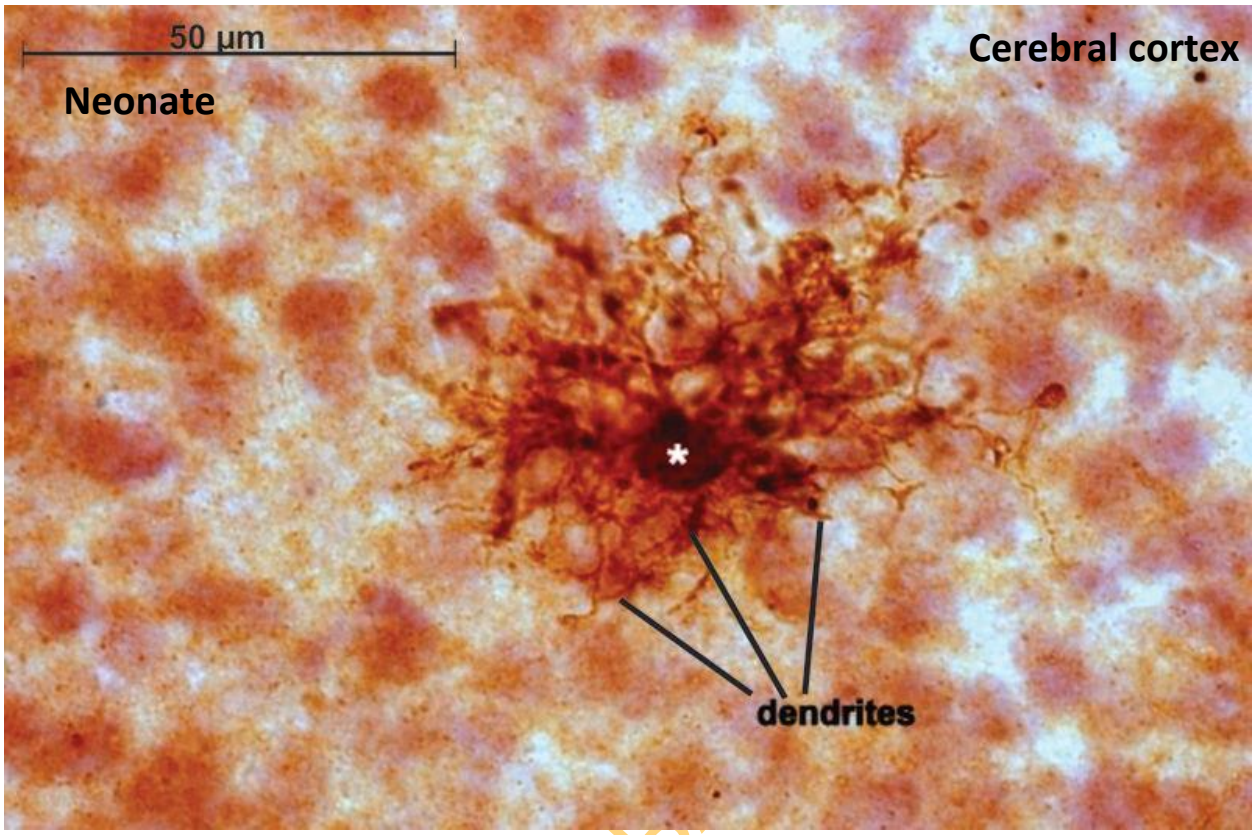


Figure 18: representative photomicrograph showing mature non myelinating oligodendrocytes under oil immersion. Note the soma (*) and tufts of dendrites (arrowheads). Scale Bar 50µ, CNPase immunostaining

Precursor-progenitor cells dominated the brain sections with the presence of some immature and mature non-myelinating Oligodendrocytes (figures 16-18). Progenitor cells were approximately 8-10µm in diameter and appeared only as cell bodies with tiny non-branching or no dendritic processes. There were pre-oligodendrocytes in sparse quantities while the immature oligodendrocytes appeared with few processes with loose dendritic extensions distinguished from the mature non myelinating cell types which had cell bodie with more prominent and extensive aborization of cell processes giving tuft-like dendrites.

Regional peculiarities were not pronounced however, it was observed that the cells of the mature non myelinating cells in the cerebellar region showed slight morphological clumping of dendrites which appeared to be less clumped in the forebrain.

In juveniles, there was evidence of myelin deposition with the presence of cell types basically consisting of the progenitor-precursor cells and the mature myelinating cell types (Figure 19). This pattern was similar to that found in adults, where the cells were overshadowed by the myelin sheath with matured myelinating cell bodies were seen intersparsed within the sheath (Figure 20-22). Cell bodies were also approximately 10µm. The cells were more numerous in the juvenile than in the adults and had more myelin sheath deposition. Kluver and Barrera sections also confirmed the myelin patterns. White matter regions of the brain showed more immunostaining intensity and myelin deposition.

Neonates demonstrated a caudo-rostral migration of oligodendrocytes from the hind brain towards the rostral migratory stream, especially along white matter tracts (Figures23-24).

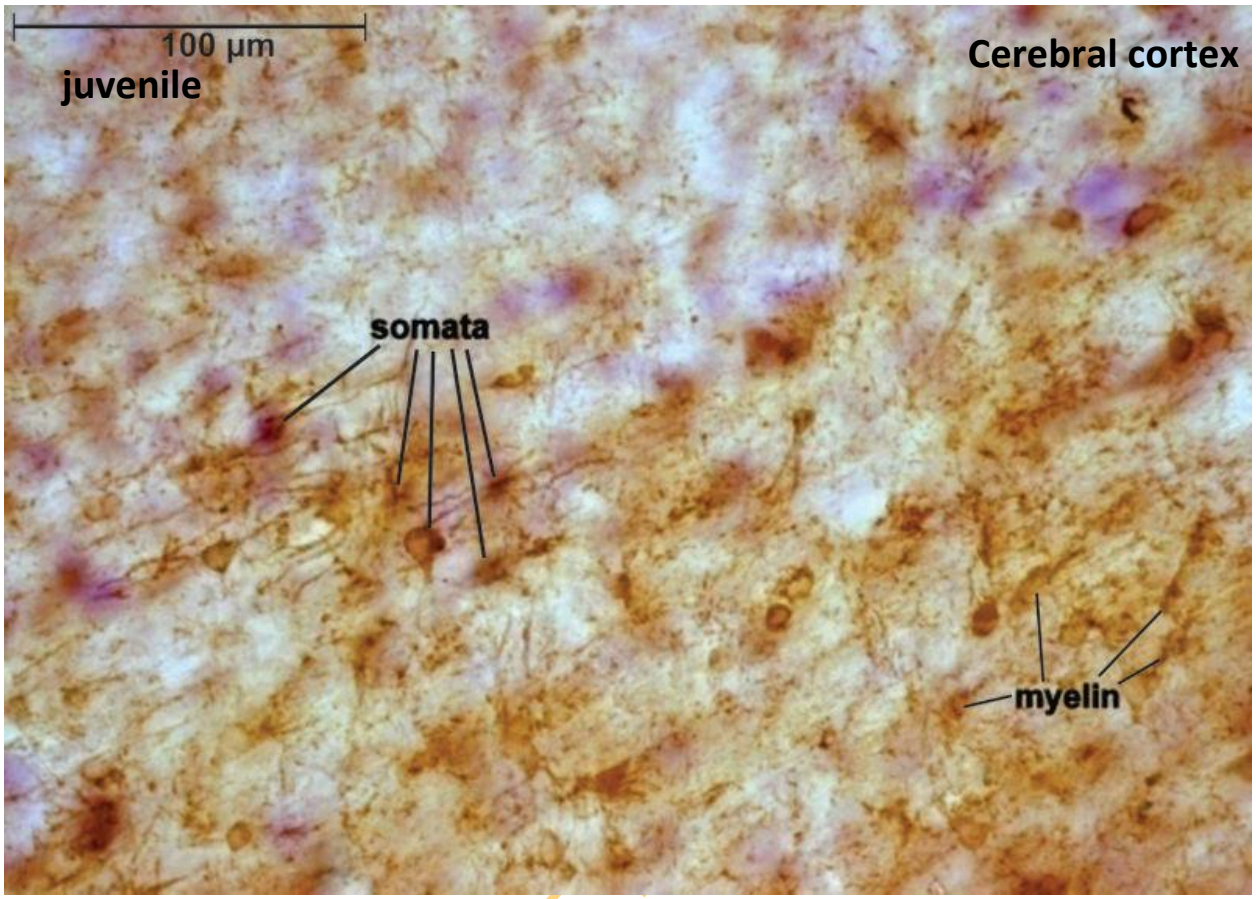


Figure 19: representative photomicrograph showing mature myelinating oligodendrocytes with myelin sheaths in the background Scale Bar 100µ, CNPase immunostaining

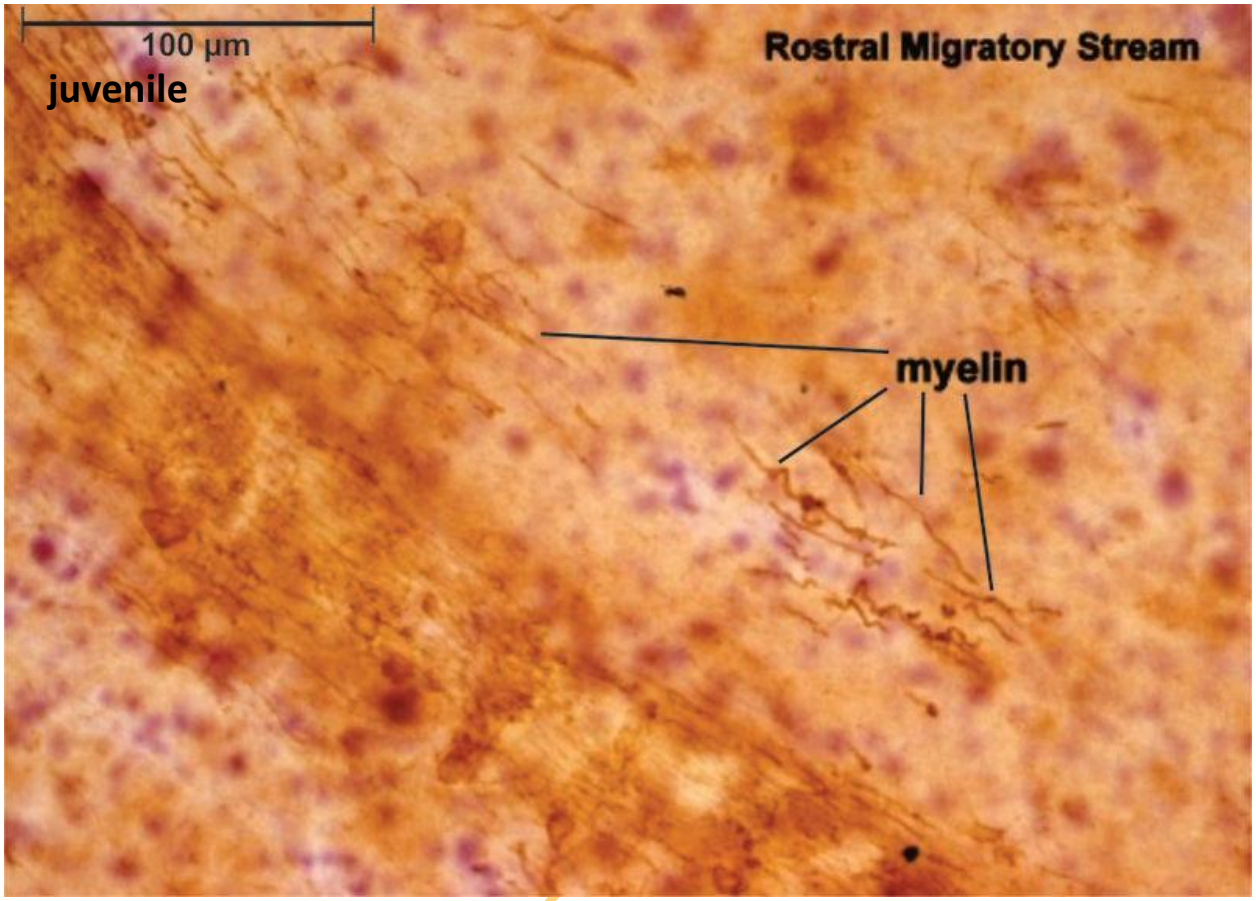


Figure 20: representative photomicrograph showing mature myelinating oligodendrocytes and evidence of myelination in the background in the olfactory bulb along the RMS Scale Bar 100µ, CNPase immunostaining

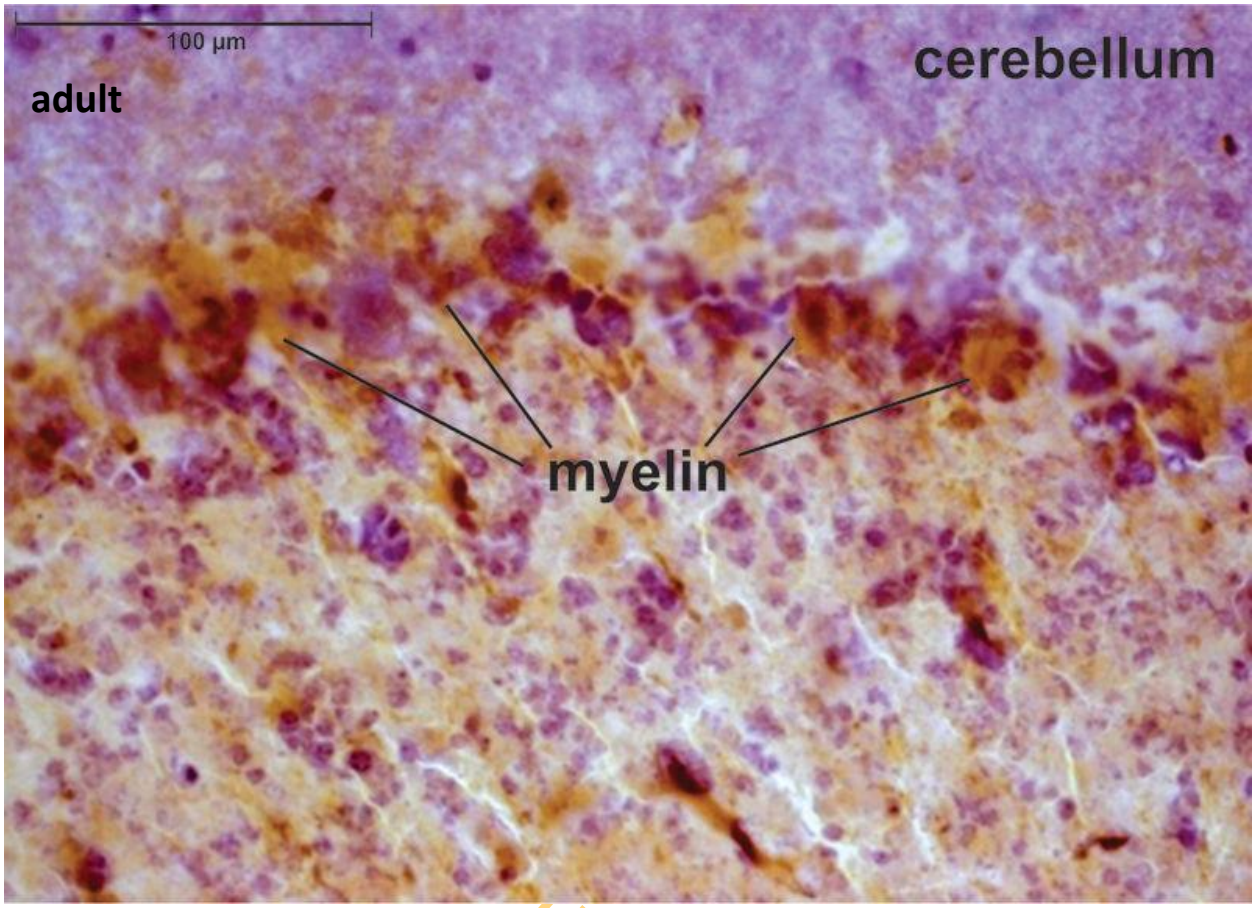


Figure 21: representative photomicrograph showing myelin deposits in the cerebellum along the Purkinje cell layer Scale Bar 100µ, CNPase immunostaining.

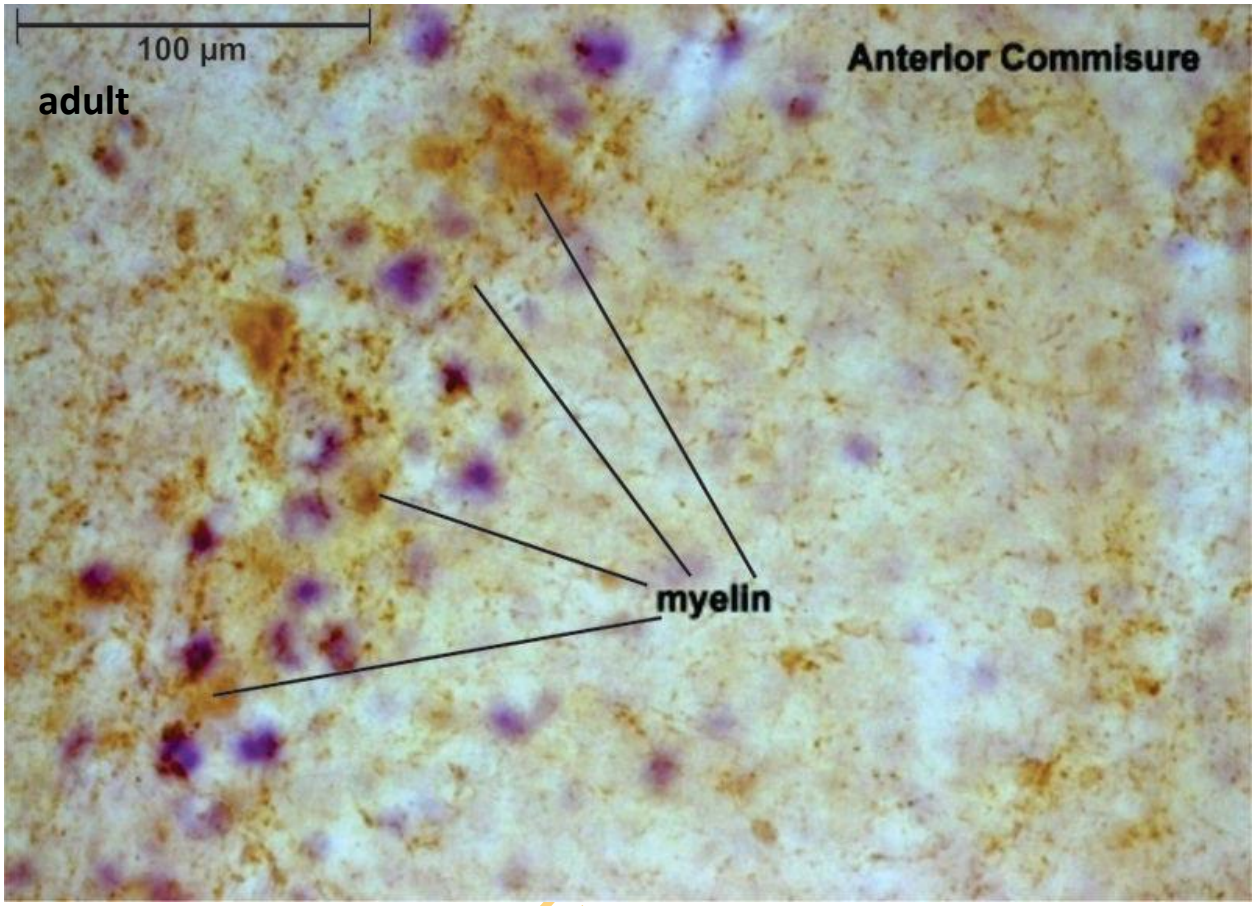


Figure 22: representative photomicrograph showing myelin deposits in the anterior commissure, Scale Bar 100µ, CNPase immunostaining

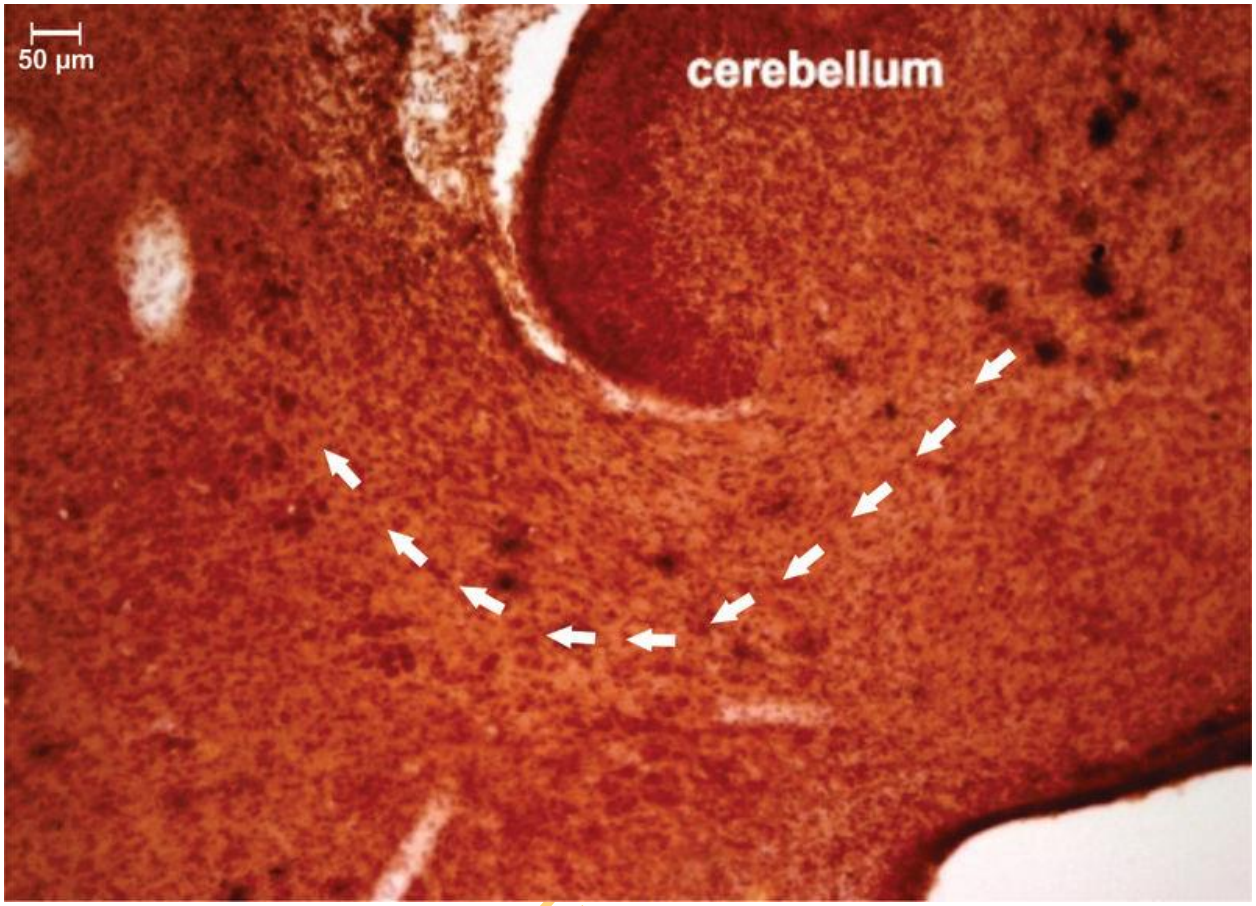


Figure 23: representative photomicrograph showing immature oligodendrocyte migration caudorostrally from the cerebellar region to the forebrain in the neonate (arrow path). Scale Bar 100µ, CNPase immunostaining

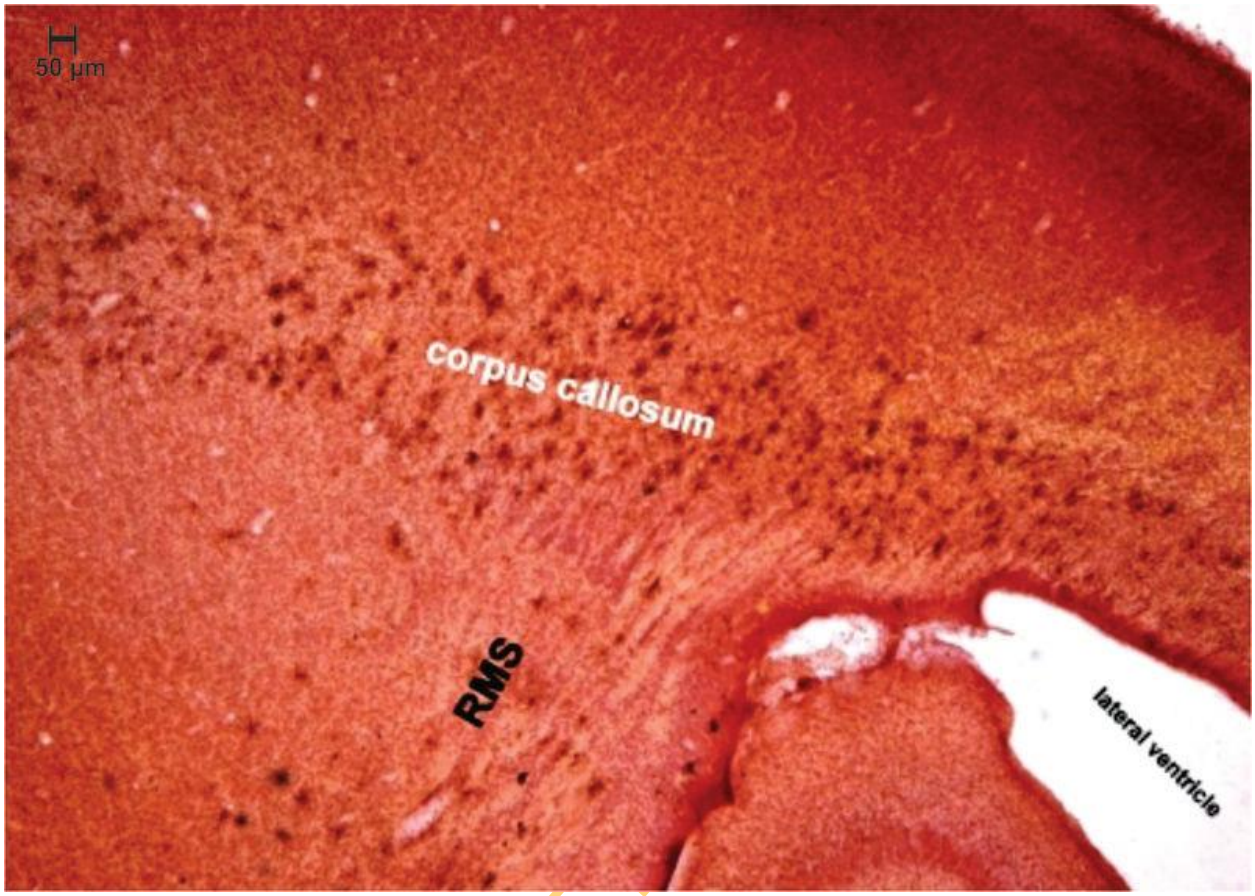


Figure 24: representative photomicrograph showing migrating oligodendrocytes in the cerebral cortex at the white matter region region of the corpus callosum, ventricle and along the rostral migratory stream. Scale Bar 50µ, CNPase immunostaining.

4.2.6 Microglia studies

Microglia are ubiquitous throughout all brain regions examined but with differences in cellular density and morphology. The most predominant density difference was between the grey and white matter with larger numbers in the grey than in the white matter **(**Figure 25**)**. in regions were the cell bodies were densely packed, for example in the hippocampus, there are fewer cells than in the surrounding neuropil in the cortex, there appeared to be a little variation in density with either depth or cortical area, and the number of cells in the cortex was similar to the hippocampus. Some regions e.g. the thalamus had sparse microglia populations but no region of the brain was totally devoid of microglia.

Positive staining was not achieved in neonatal subjects used in this study but juvenile and adults stained with greater intensity in the juveniles. Cell populations were typically clumped and indistinct unlike the other glia cells and thus only a qualitative description is given in this section of the study.

Two classes of $Iba1^{+}$ cells were observed in the brain of adult and juvenile AGR. First, cells found on the ventricular surface of the choroid plexus/ ventricular surface of ependymal cells-These cells have ovoid nuclei with average diameter of 9.3µm (range: 7.6-12.5 µm) and large stout processes also being found in close association with blood vessels (Figure 25).

Second class was found within the nervous tissue proper in both grey and white matter. These cell have 6.2 µm (range: 6-7 µm), little cytoplasm, but several long processes which also branch a number of times. The processes appear crenellated and covered in fine protrusions (Figures 26-27). The morphological geometry however seemed to vary depending on location. Cells in the grey matter had predominantly radiate morphology while those in the white matter had processed oriented along the long axis of the fibre tracts. These cells appeared to have their own domains as no intertwining was observed

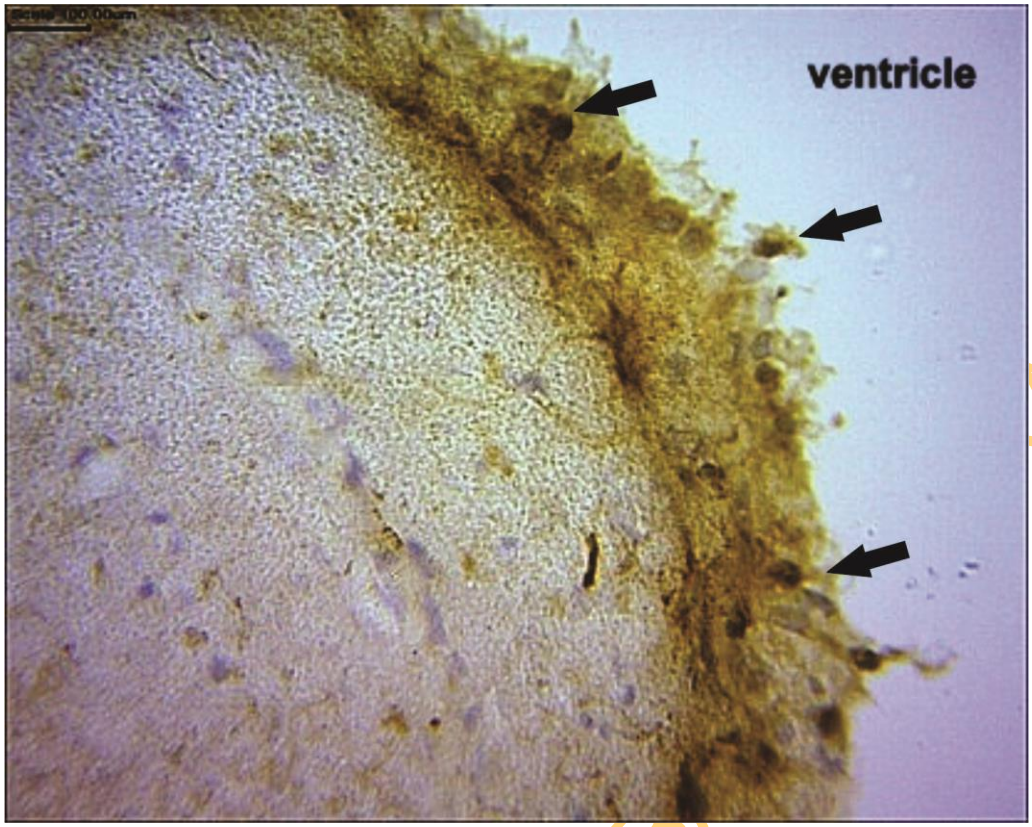


Figure 25: representative photomicrograph showing type I ventricular microglia in the ventricular region of juvenile AGR. IbA-1 stain, Scale bar-100µm

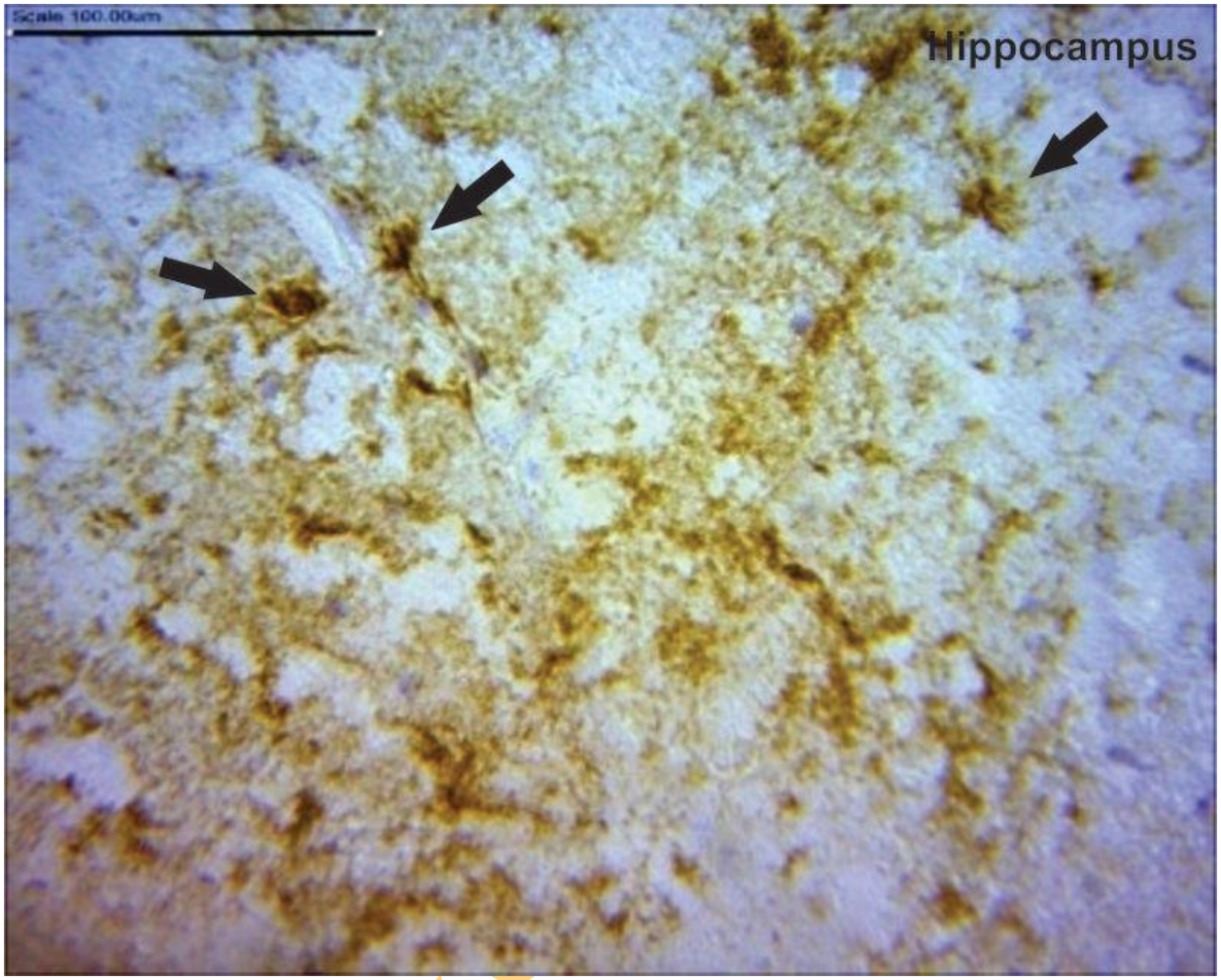


Figure 26: representative photomicrograph showing type 2 microglia in the hippocampal region of an adult AGR brain IbA-1 stain Scale bar-100µm

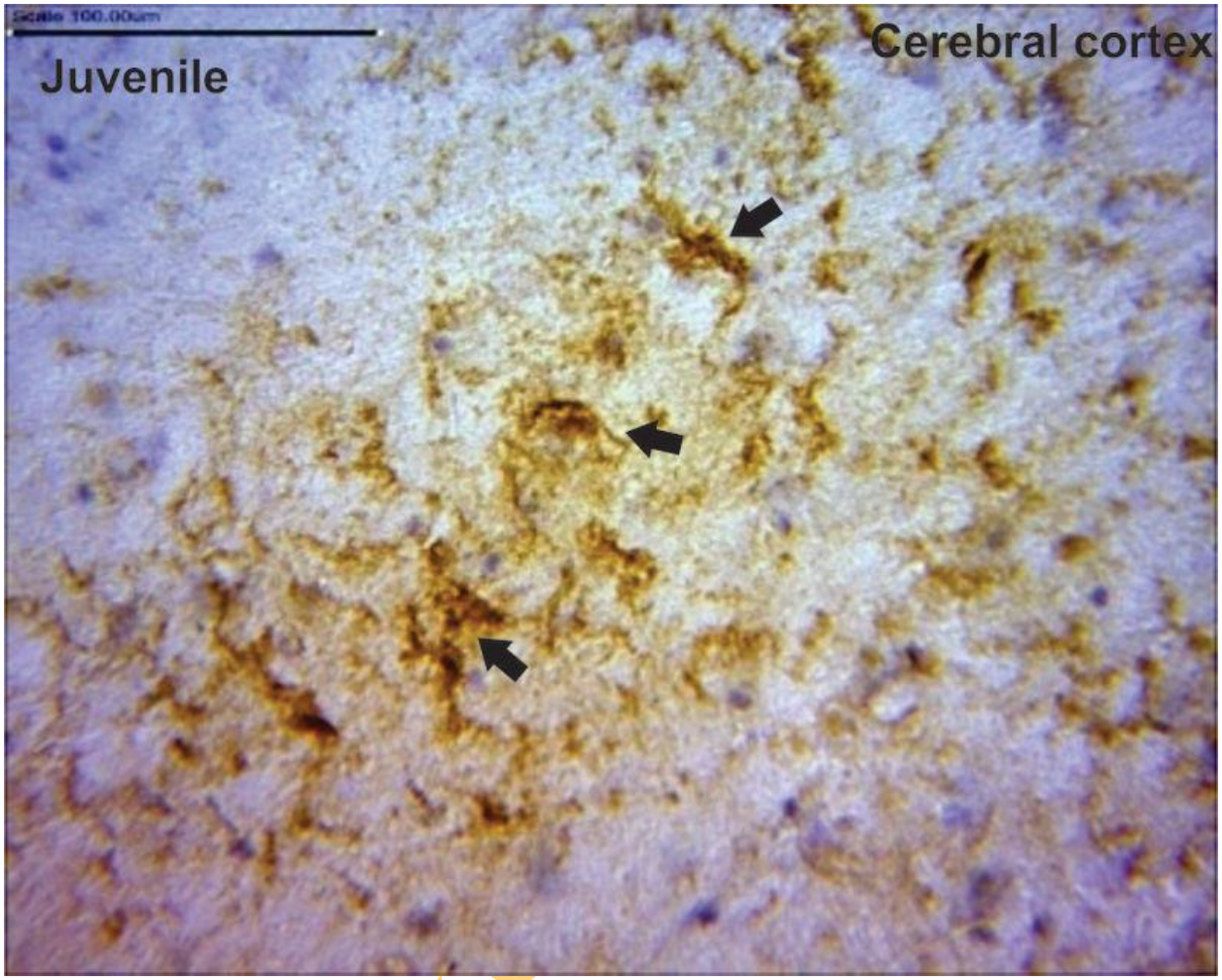


Figure 27: representative photomicrograph showing type 2 microglia in the cerebral cortex of juvenile AGR brain. IbA-1 stain, Scale bar-100µm

4.2.7 General Laminar organization of the Cerebellum

The cerebellum was made up of a cortex of grey matter and a core of white matter containing four pairs of nuclei. Afferent and efferent fibres pass to and from the brain stem and rostral cerebellar peduncles via the caudal middle and rostral cerebellar peduncles linking the medulla, pons and midbrain respectively. The cortex formed a series of deeply convoluted folds (folia) supported by a branching central medulla of white matter. The cerebellum was observed to consist of 3 basic layers (molecular, Purkinje and granular layers) in the juveniles and adults. However, the neonates had a typical superficial external granular layer making up its fourth layer. This layer is known to disappear within the first few weeks of postnatal life. However, an unreported persistent mantle of the external granular layer was observed particularly in juveniles up to adult life in the AGR. In neonates, the thickness of this layer was found to be 14.30 ±1.5µm.

Other differences found were in thickness of layers and number of Purkinje cells found within defined anatomical regions. Molecular cell layer measured an average of 247.40 ±30.15µm, 254.81± 25.27µm and 19.47±3.06 µm, in adults, juveniles and neonates respectively. The Purkinje cells layers measured 36.63±7.40 µm, 37.81±1.41 µm and 6.56 ±0.98 µm in adults, juveniles and neonates respectively. They had cell bodies of (24.03 ± 1.13 µm, 23.93± 1.01µm and 4.53 ±1.26 µm) in adults, juveniles and neonates respectively, a relatively fine axon extending down through the granule cell layer and an extensively dendritic system which aborized into the outer molecular layer (Figure 28). Granule cell layer was largest in adults 286.20 ± 10.39µm, juveniles had a thickness 240.50 ± 6.08 µm while neonates 35.33±4.40µm.

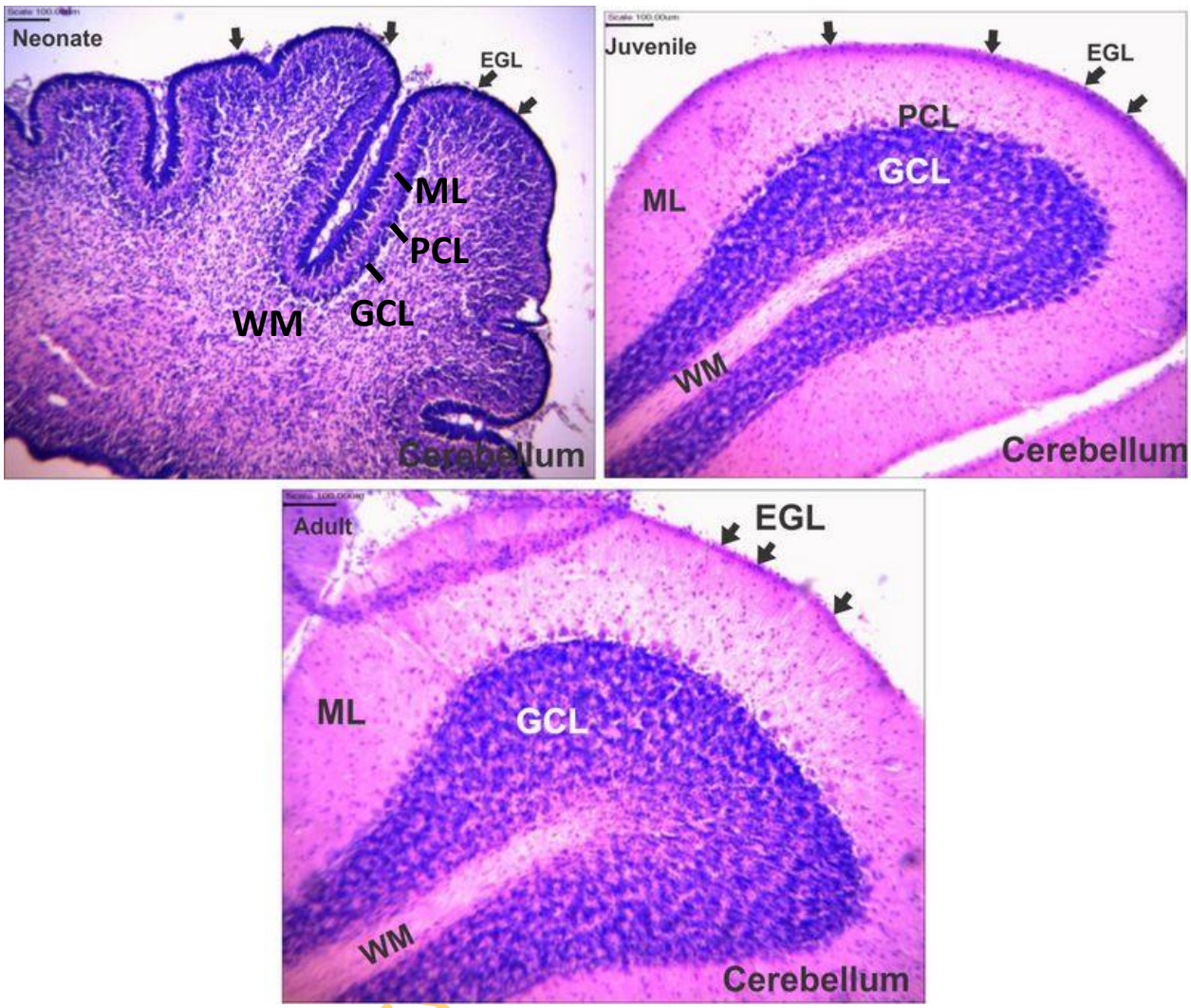


Figure 28: comparative representative photomicrographs showing the cerebellum in the AGR across the age groups. Note the persistent mantle layer of the external granular layer (arrows) in juvenile and adult cerebellum. EGL= external granular layer, ML=molecular layer, PCL=Purkinje cell layer, GCL=granule cell layer, WM=White matter. H&E, Scale bar-100µm

4.2.7.1 Histomorphometry of the Cerebellum

Table 4.6: Histomorphometric values of the cerebellum

	Age			Anova	
	Neonate	Juvenile	Adult	F	P- value
EGL	14.30 ± 1.53	----	----	---	-----
ML Thickness	19.47 ± 3.06	254.81± 25.27	247.40 ± 30.15	99.81	0.0001
PCL Thickness	6.56 ± 0.98	37.81 ± 1.41	36.63 ± 7.40	47.41	0.0001
Purkinje Diameter	4.53 ± 1.26	23.93 ± 1.01	24.03 ± 1.13	351.06	0.0001
Purkinje Density	6.67 ± 0.58	3.8 ± 0.45	3.00 ± 0.00	89.41	0.0001
GL Thickness	35.33 ± 4.40	240.50 ± 6.08	286.20 ± 10.39	1012.61	0.0001

Juveniles had the highest ML thickness (254.81± 25.27) followed by adults (247.40 ± 30.15) and neonates (19.47 ± 3.06). Similar pattern was observed in the variation of PCL thickness and GL thickness among the age groups. However, highest Purkinje diameter and density were found in adults and neonates respectively.

4.2.8 General laminar organization of the main olfactory bulb

The olfactory bulb of the AGR appeared as a bulbous elongation from the rostral portions of the cerebrum (Figure 29). Microscopically, it was divided into the MOB and a dorsally located accessory olfactory bulb (AOB). The layers of the olfactory bulb as demonstrated by cresyl violet staining for Nissl bodies and Kluver and Barrera staining for myelinated fibres and H and E (Figure 30a-c) revealed 6 layers of the MOB viz: the olfactory nerve layer (ONL), the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), the granule cell layer (GCL) and the periventricular layer (PVL). The EPL and GCL layers were further subdivided into inner and outer sub layers with clear variations in the cellular patterns (Fig.30b).

4.2.8.1 Olfactory nerve layer

This was presented as the most superficial layer of the MOB and stained cell bodies across with cresyl fast violet and showed a slight degree of myelination with K and B **(**Figure 30c**)** which was confirmed by CNPase immunopositive oligodendrocyte soma and processes with evidence of some myelin sheath deposition **(**Figure 31**)**. GFAP immunoreactive cell bodies were randomly organized and had their processes greater in number than the cell bodies (Figure 31).

4.2.8.2 Glomerular layer

The 1-2 layered glomeruli were typified by large spherical glomeruli (averaging 40 µm in the neonates and between 130-150µm in diameter in juveniles and adults). K&B staining was positive for myelin sheath fibers in the glomerular and periglomerular regions. Oligodendrocytes soma was seen along the periglomerular area with axonal extensions and myelin deposition (Figure 31c-d). GFAP immunohistochemistry revealed cell bodies and processes within the periglomerular area (Fig.31a-b). Mid centre of the olfactory bulb in adults and juveniles in particular, the GL had 6-10 layers which was absent in other regions of the OB. This might be largely responsible for the keen sense of smell in the AGR

4.2.8.3 External plexiform layer

This layer was found ventral to the glomerular layer and was further divisible into inner and outer layers. Myelin fibers were positively stained and further confirmed by the presence of soma and dendrites of oligodendrocytes with myelin sheath deposition using CNPase immunostaining **(**Figure. 31c-e). GFAP positive cells were sparse in this layer but present.

4.2.8.4 Mitral cell layer

This layer appeared as a band of mitral cells one to two layers thick (Fig30 a-c) with cresyl violet stain and no evidence of myelin staining with K&B as also confirmed by absence of soma and sheaths with CNPase immunostaining. GFAP positive cells were sparse and tangentially arranged.

4.2.8.5 Granule cell layer

The relatively thick GCL was made up of both large and small granules which divided the layer into 2 cluster zones of inner and outer GCL with intense staining (Figure 30). K and B diffusely stained the oligodendrocyte soma and myelin sheath as observed widely with CNPase immunostaining. The GFAP immunoreactivity resembled those of the EPL but cells were randomly oriented.

4.2.8.6 Periventricular layer

Nissl stain revealed an extensive PVL which extended towards the RMS (Figure 31a**)**. K and B for myelin was also widely present as confirmed by CNPase positive cells which were observed to be similar to those of the GCL as it moved towards the RMS. GFAP positive cells with increasingly large numbers of glial processes were observed and randomly oriented in this layer.

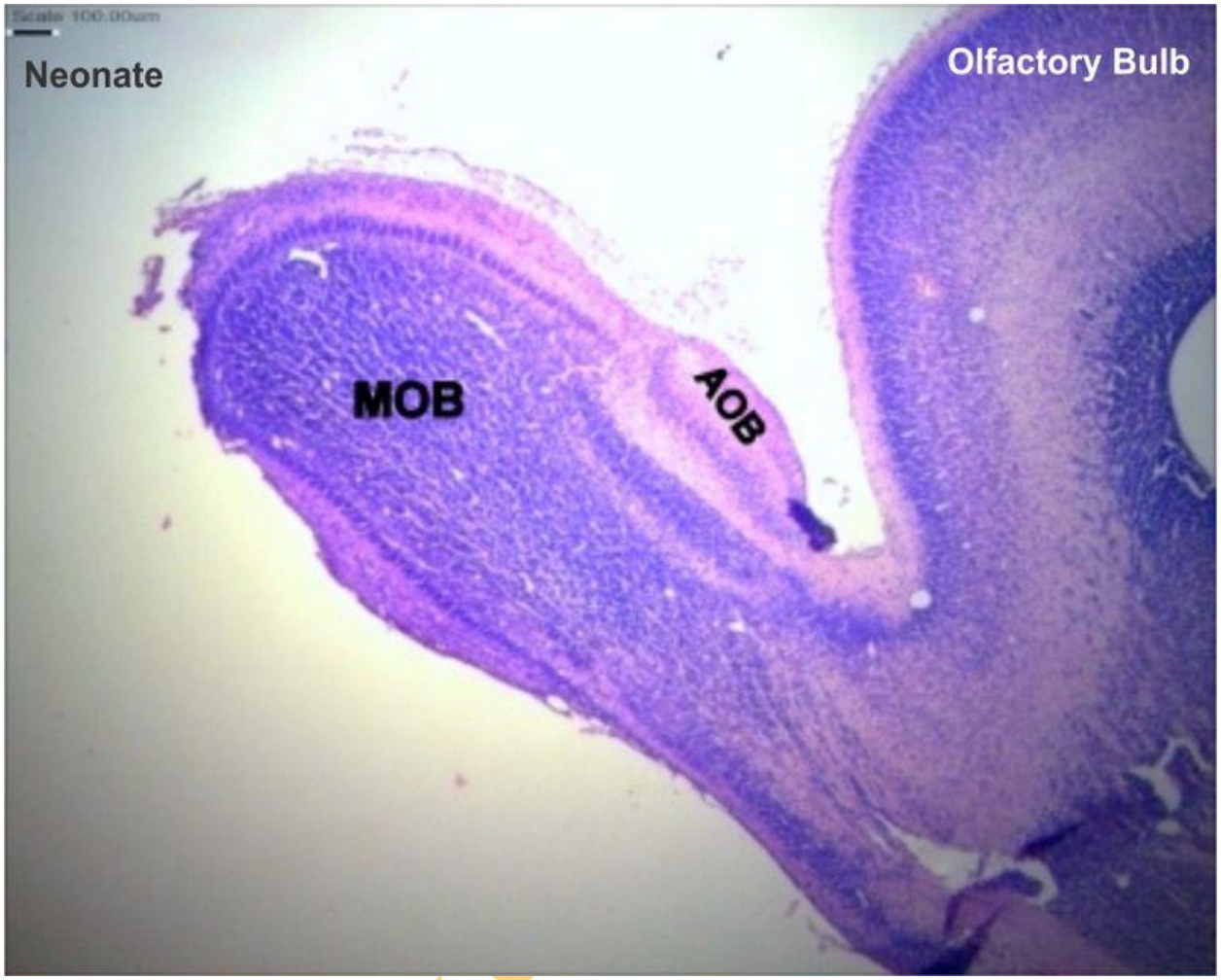


Figure 29: representative photomicrograph showing the olfactory bulb in the neonate AGR. AOB=accessory olfactory bulb, MOB=main olfactory bulb. H&E, Scale bar-100µm

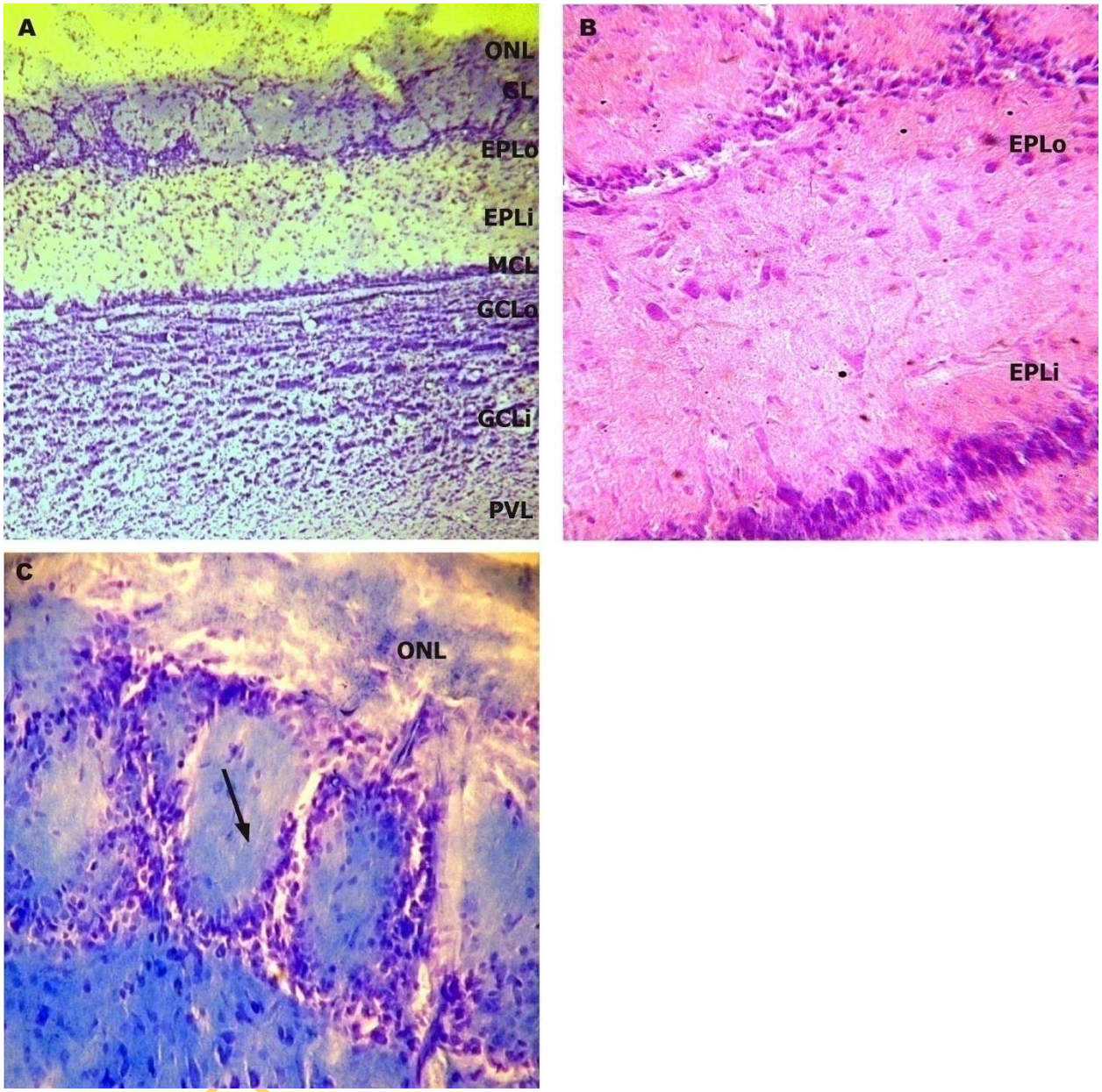


Figure 30: Representative Photomicrographs of (A) Nissl stained section from the MOB showing the layers of the MOB in the African giant rat (B) H and E section revealing the cellular differentiation of the EPLo and EPLi (C) Kluver and Barrera staining showing myelinated fibres (light blue) within the glomerular and external plexiform layers (arrows). Note the relative absence of myelination in the ONL. Scale bar-100µm

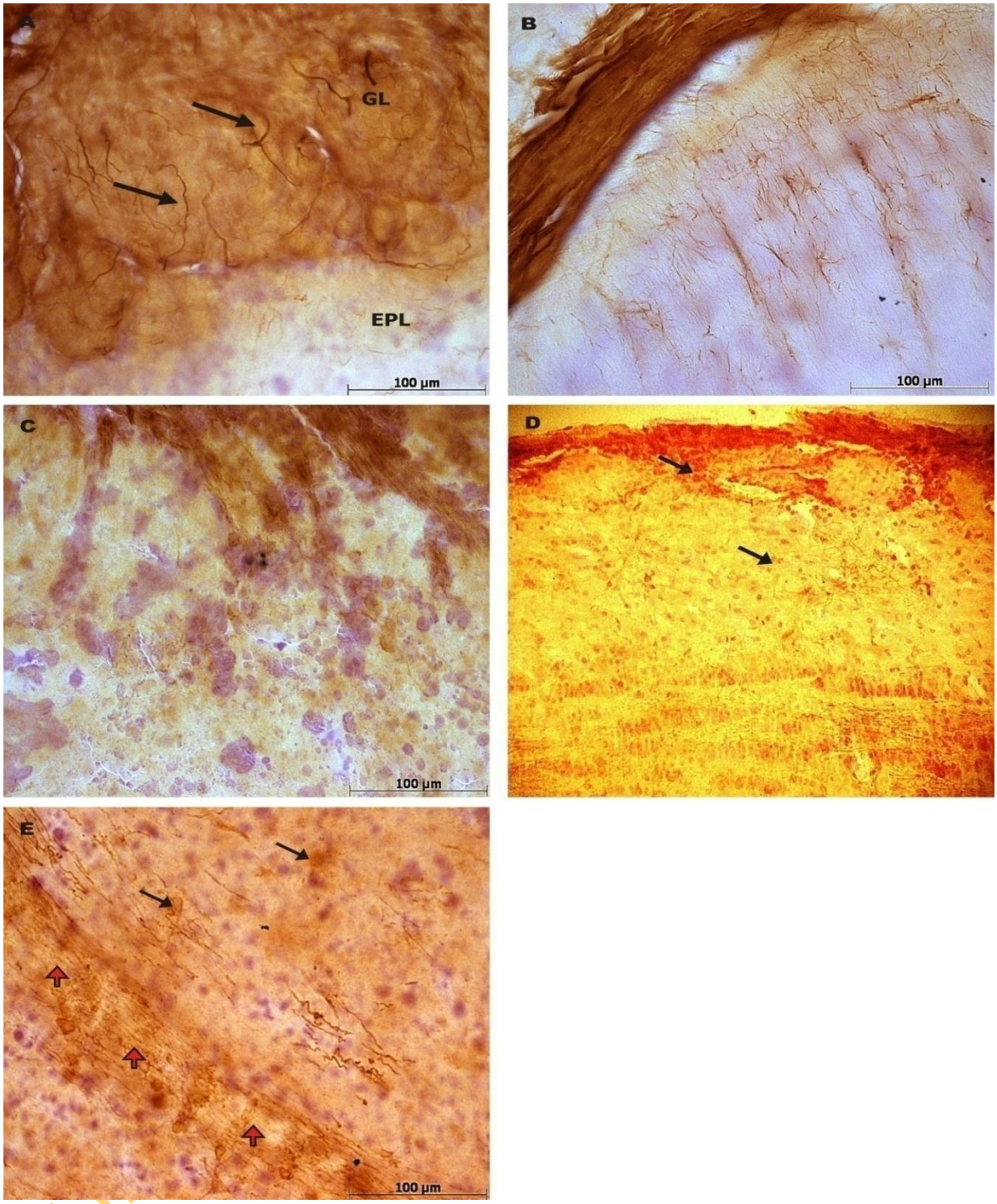


Figure 31. Representative micrographs of immunostaining for glia showing (A-B) GFAP+ processes in the glomerular and periglomerular regions (processes appear to pass through the glomerular neuropil (arrows) extending from the extensive ONL, extending distally towards the granular layer of the Olfactory bulb (C-D) CNPase+ staining in the ONL and periglomerular layers (E) oligodendrocyte soma with evidence of myelin sheath deposition (arrows). Scale bar-100µm.

4.3 HISTOMORPHOMETRIC STATISTICS

Table 4.7: Mean ± SD of the different olfactory bulb layers based on age.

Olfactory bulb layer	Age			ANOVA	
	Neonate	Juvenile	Adult	F	P- value
Glomerular layer	46.18 ± 7.28	138.43 ± 28.86	131.56 ± 32.82	20.18	0.0001
External plexiform layer	49.68± 19.54	455.72± 53.26	311.71 ± 25.33	164.69	0.0001
Mitra cell layer	27.66 ± 4.15	88.02 ± 22.19	31.12 ± 8.05	30.00	0.0001
Granule cell layer	176.15 ± 35.52	1079.83 ± 132.66	561.79 ± 28.60	156.74	0.0001
Periventricular layer	187.48 ± 10.69	904.82 ± 237.74	528.85 ± 85.56	30.20	0.0001

The table above shows the influence of age on the size of olfactory bulb layers of the African giant rat. In all the layers of olfactory bulb, juveniles have larger diameter compared to both adult and neonate. This mean difference in olfactory bulb layer diameter among the age groups of African giant rat is statistically significant indicating that it was not due to sampling error or any form of bias . Among the neonates, the periventricular layer has the largest diameter (187.48 ± 10.69) while the mitra cell layer has the least (27.66 ± 4.15). However, among the more matured rats, the granule cell layer has the largest diameter while the mitra cell layer has the least.

The LOB lengths were6.43±0.24cm, 7.85±0.57cm and 3.25±0.19cm while the ROB lengths 6.49±0.33cm, 7.51±0.57cm and 3.13±0.24cm for adults, juveniles and neonates respectively.

4.4 ADULT NEUROGENESIS STUDIES

4.4.1 Immunohistochemistry

Summary of all sites of immunoreactivity as listed in Table4.8 shows the profile of adult neurogenesis in the brain of wild caught AGR across defined age groups using the proliferative marker Ki-67 and immature neuron marker DCX. With the antibody Ki-67, proliferating cells are usually detected only during the active phases of the cell cycle. Ki-67 immunoreactivity was apparent in the active sites with nuclei of cells located in the SGZ of the dentate gyrus of the hippocampus, the granular, sub-granularcell layer and hilus of the DG (Figs 32,34,36) and the subventricular zone (Figs 33, 35, 37). These appeared in clusters and widely distributed with darkly stained cells and centrally located darkly stained nuclei. In the active sites, higher densities of proliferative cells were observed in the juvenile than in adults and were mostly visible around the SVZ and towards the RMS. The neonate brains showed a widespread distribution in practically all brain regions (Fig. 38). The juvenile brains however showed immunopositive cells in potential sites of the brain including the cerebral cortices (Figures 39) and the cerebellum; these features were absent in all adult brains investigated.

4.4.2 Localization of DCX-Expressing Cells

In all age groups, DCX-immunopositive cells were observed in several regions of the brain, namely, the DG, SVZ, RMS, OB, anterior commissure, striatum, the somatosensory and Piriform cortices. Neonate brains showed intense DCX-labelled cells (Figure 38). Cell morphology was often hard to define, especially in the neonate brain, where labelled cell bodies were densely packed and interspersed with DCX-labelled processes.

Positive cells were present along the whole extension of the SVZ towards the RMS and DG (Figures 40-41) in juvenile samples which showed types E and F classified cells features which were greatly reduced in quantity in adult samples (Figures 43) with only type E subgranular cells. The DCX positive juvenile cells were observed along the whole RMS with densely packed, small round or spindle-shaped somas and the majority of them had two processes. In the DG of all juvenile and adult groups, DCX labeled cells and processes were present in the sub granular zone of the hilus and granular layer of the DG. In the granular DG layer, most labelled cells had large somata with well-differentiated apical dendrites extending to the molecular layer and branching there. The juvenile brains had densely stained clumps of neurons with somata in the sub granular layer and dendrites extending over the granular layer

into the hilus of the dentate gyrus, staining was also observed along the mossy fibers. The adult AGR only showed slightly less immunopositivity strictly in the SVZ and DG. DCX-immunopositive spindle-like cells were found migrating through layer III towards layer II of the piriform cortex and in the somatosensory cortex, with cells migrating along the RMS with small, round somata and either one or two short processes extending perpendicularly to the surface of the cortex (Figures 47-50) DCX positive cells were seen occasionally in the striatum and anterior commissure of some juvenile species (n=2) but not in the adult.

4.4.3 Proliferative Cell Counts

Ki-67 positive cells were counted to estimate total proliferative cells in the SVZ and DG. Juvenile rats had the highest values 45530±13950 and 12480±7860) (while adult counts were 6880±340 and 1130±150 in the SVZ and DG respectively. Neonates had (21145±8395 and 11800±1230) (Figure 51-52).

Correlative studies

Table4.8: The mean and standard error of mean (SEM) of body and brain weights of the animals used in the study

	Body weight (grams)	Brain weights (grams)
Neonates	18.68±0.69	0.40± 0.00
Juveniles	300±36.51	4.48±0.18
Adults	975±40.31	5.48±0.23

Table4.9: Summary of investigated age groups and qualitative immunohistochemical results using the proliferative marker Ki-67 and the immature and migrating neuron marker DCX (+ indicates presence; - indicates absence, +/- indicates indecisive and more than one "+" indicates increasing level of immunopositive staining).

	NEONATES		JUVENILES		ADULTS	
	Ki-67	DCX	Ki-67	DCX	Ki-67	DCX
DG/HIPP	++	+++	++	++	+	+
SVZ	++	+++	++	++	+	+
RMS	++	+++	++	++	+	+
CORTEX	++	+++	++	+	-	-
ANT COMM	++	+++	-	±	-	-
STRIATUM	++	++	±	-	-	-
CEREBELLUM	++	++	±	-	-	-

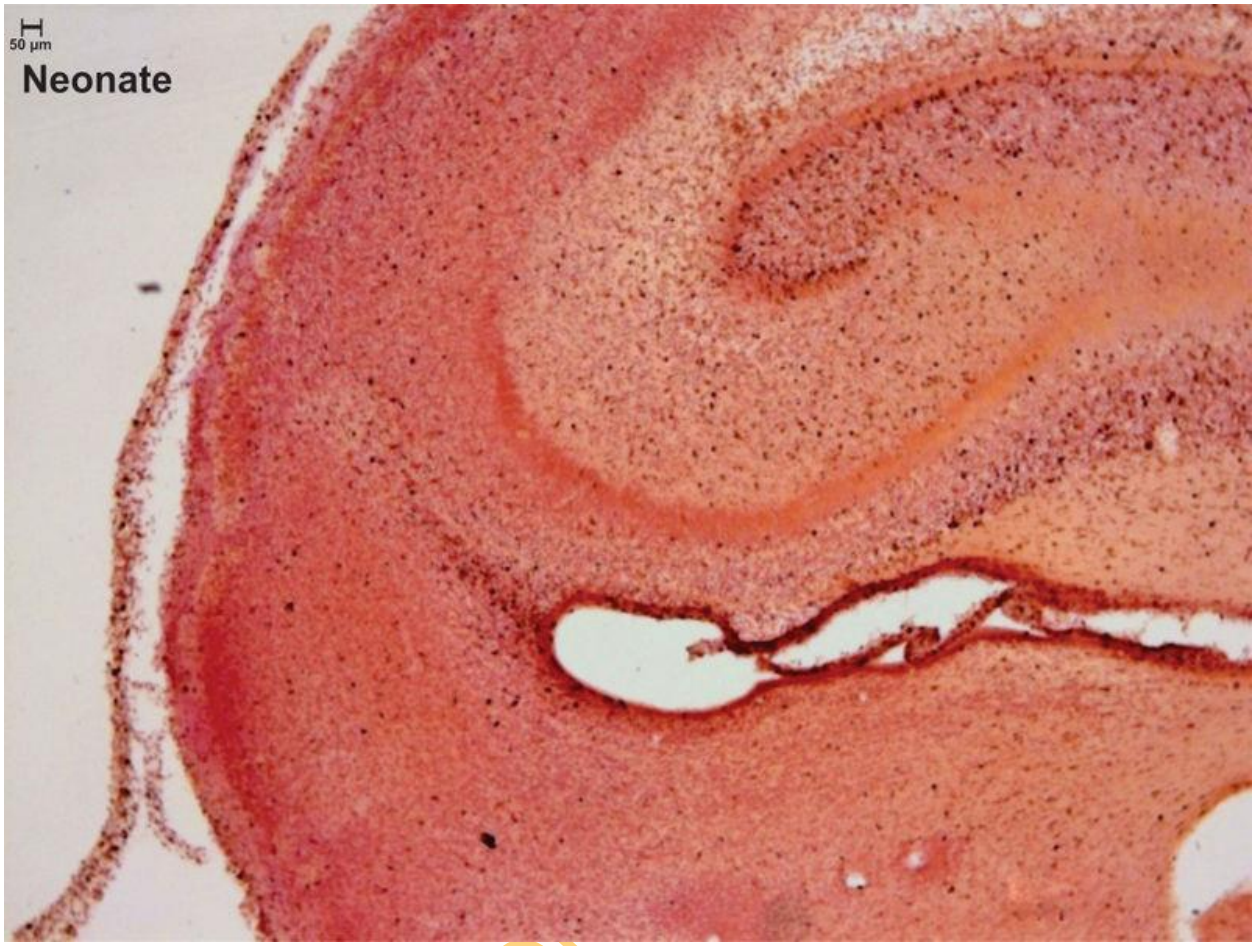


Figure 32: representative photomicrograph showing Ki-67 immunopositive cell in the neonate AGR across various regions of the brain including the hippocampus and cortex.

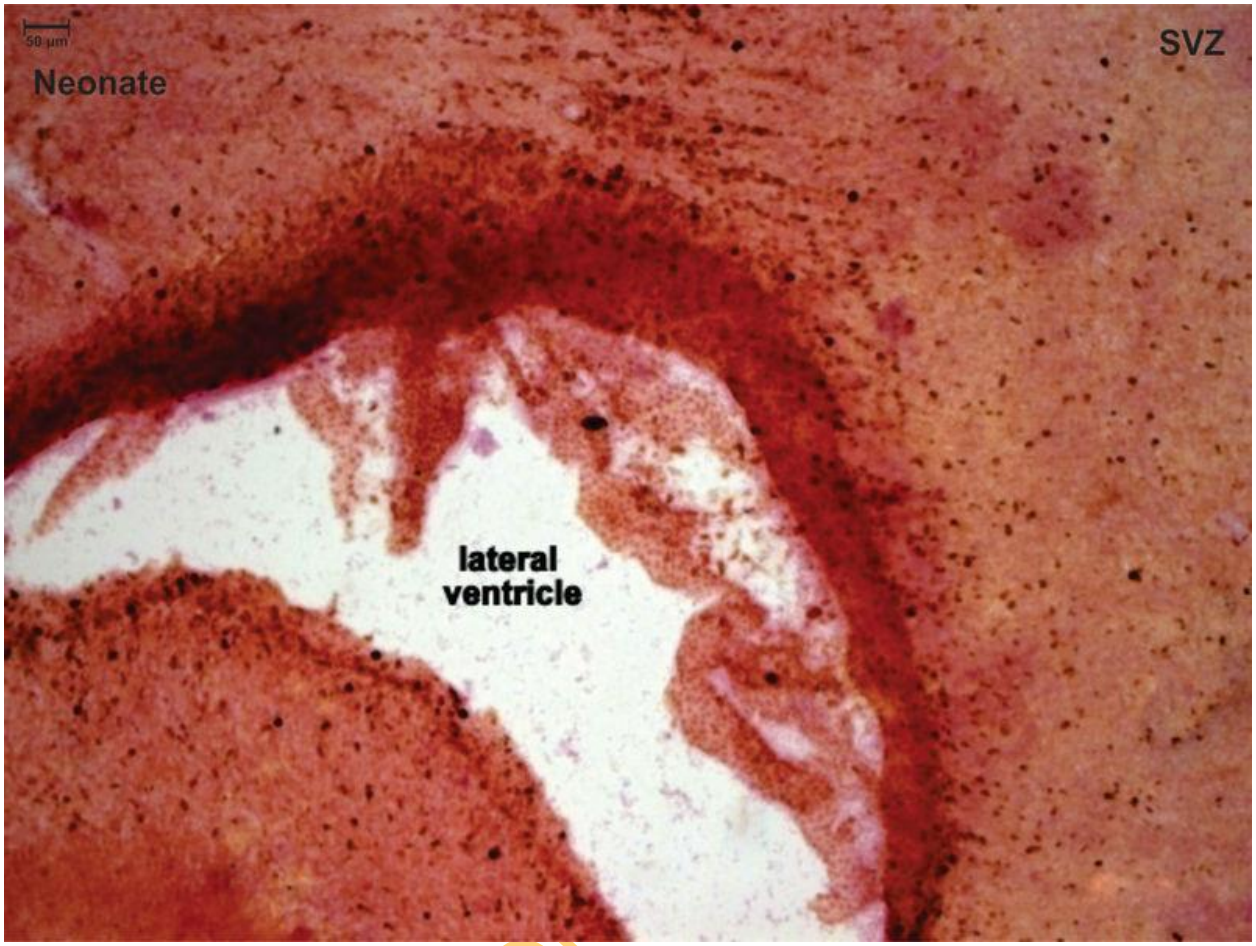


Figure 33: representative photomicrograph showing Ki-67 immunopositive cells in the subventricular zone of the neonate AGR. Scale bar-50µm

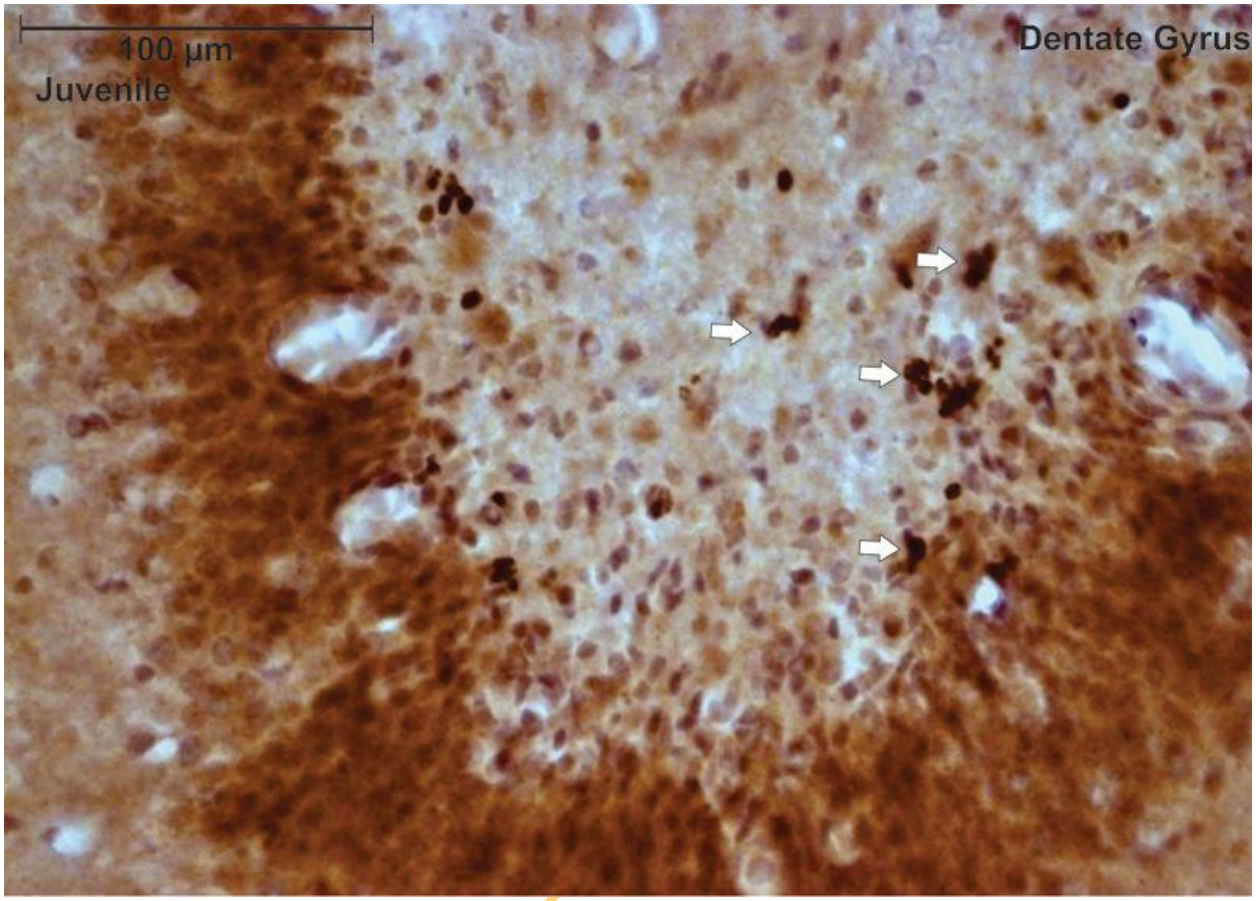


Figure 34: representative photomicrograph showing intense staining in the dentate gyrus of the hippocampus in the juvenile AGR. Numerous proliferative cells as darkly stained clusters at the sub-granular zone (arrows). Ki-67 stain, Scale bar-100µm

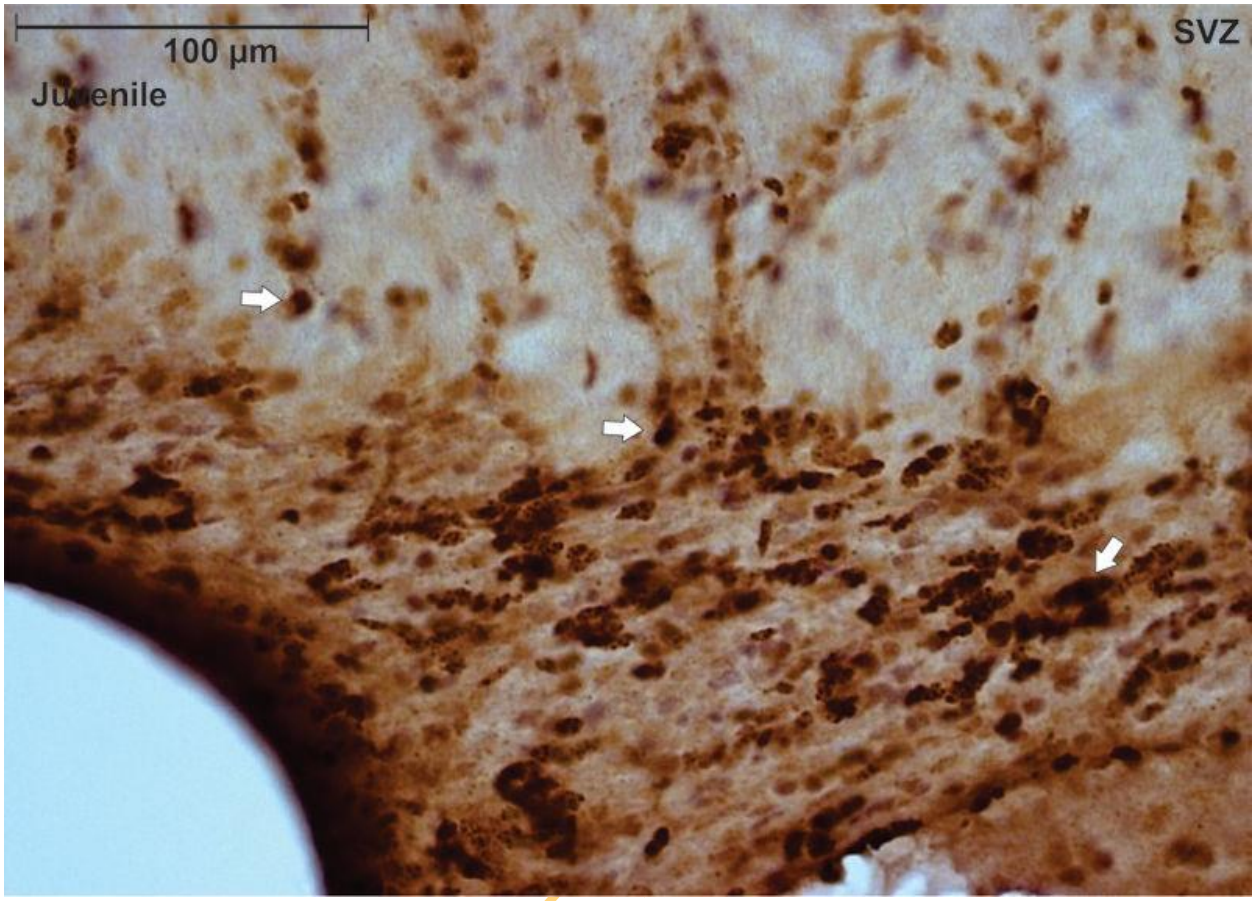


Figure 35: representative photomicrograph showing the subventricular zone of the juvenile AGR. Numerous immunopositive cells indicating neurogenesis are shown migrating towards the rostral migratory stream. Scale bar-100µm

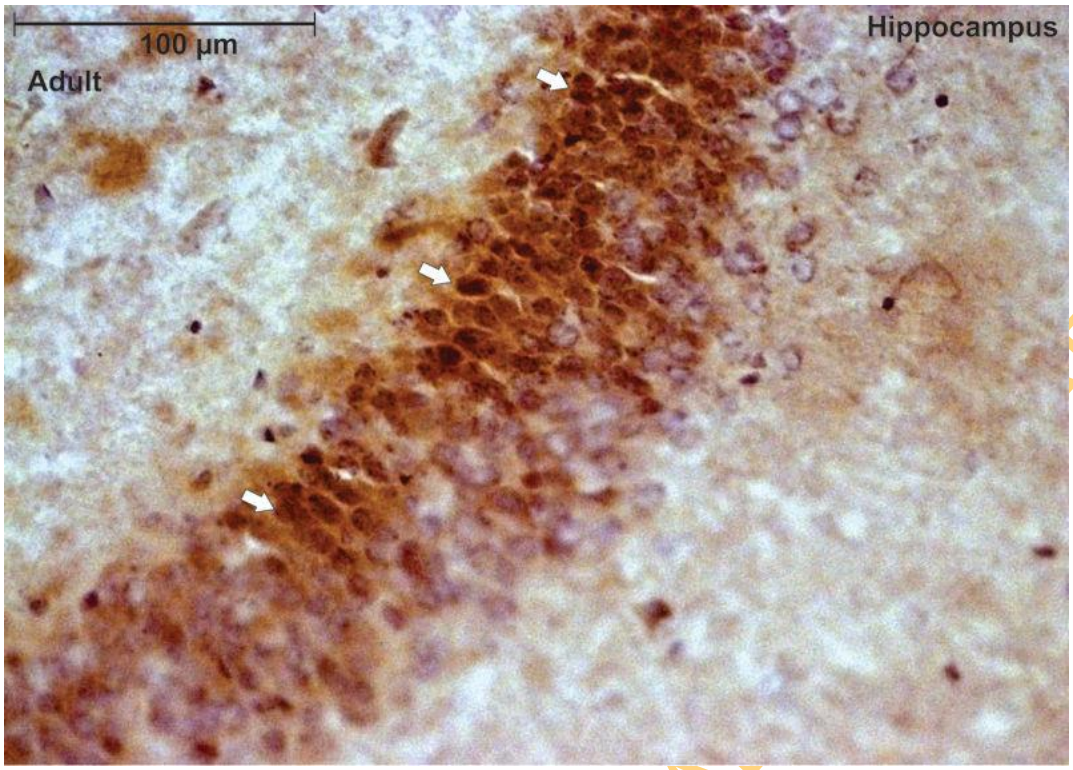


Figure 36: representative photomicrograph of adult Ki-67 sections with less intense immnostaining in the subgranular zone of the hippocampus. Scale bar-100µm

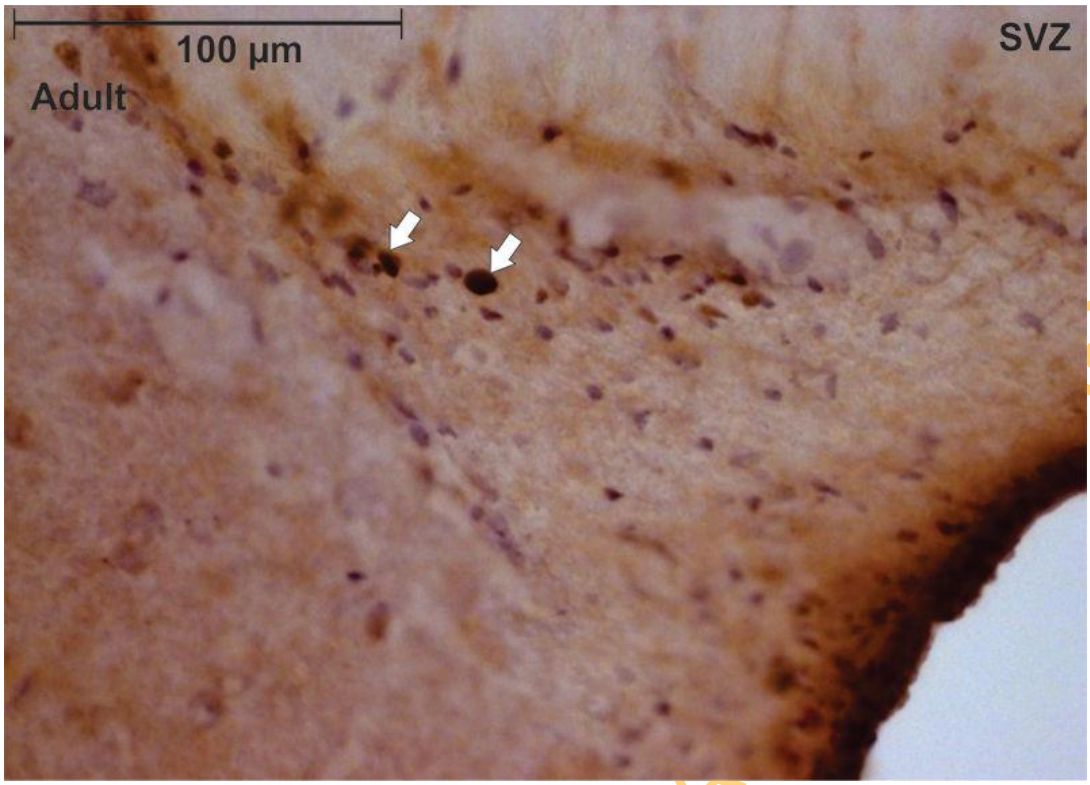


Figure 37: adult Ki-67 sections with less intense immnostaining in the subventricular zone towards the rostral migratory stream, Scale bar-100µm

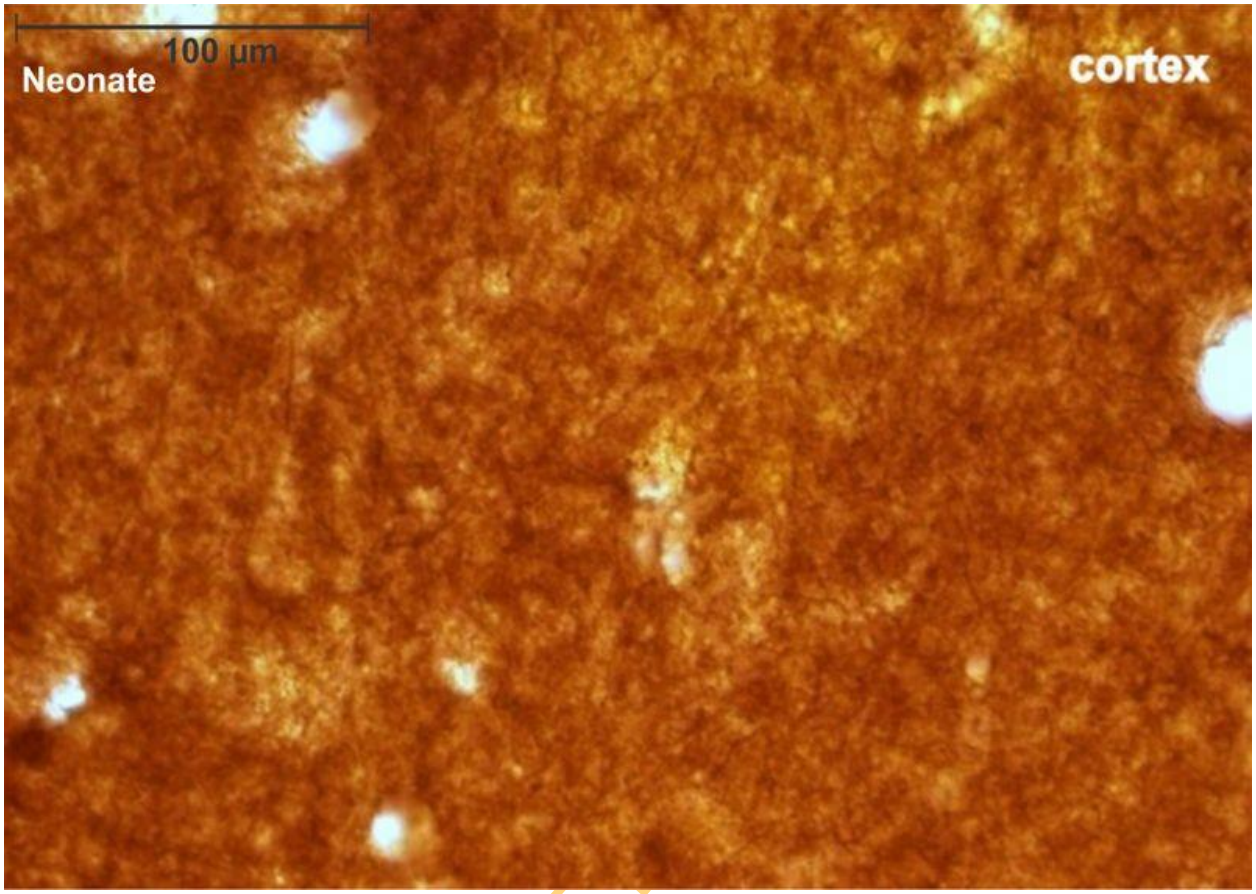


Figure 38: representative photomicrograph showing DCX imunostaining in neonate AGR with intense immune-signaling in all brain regions. Scale bar-100µm

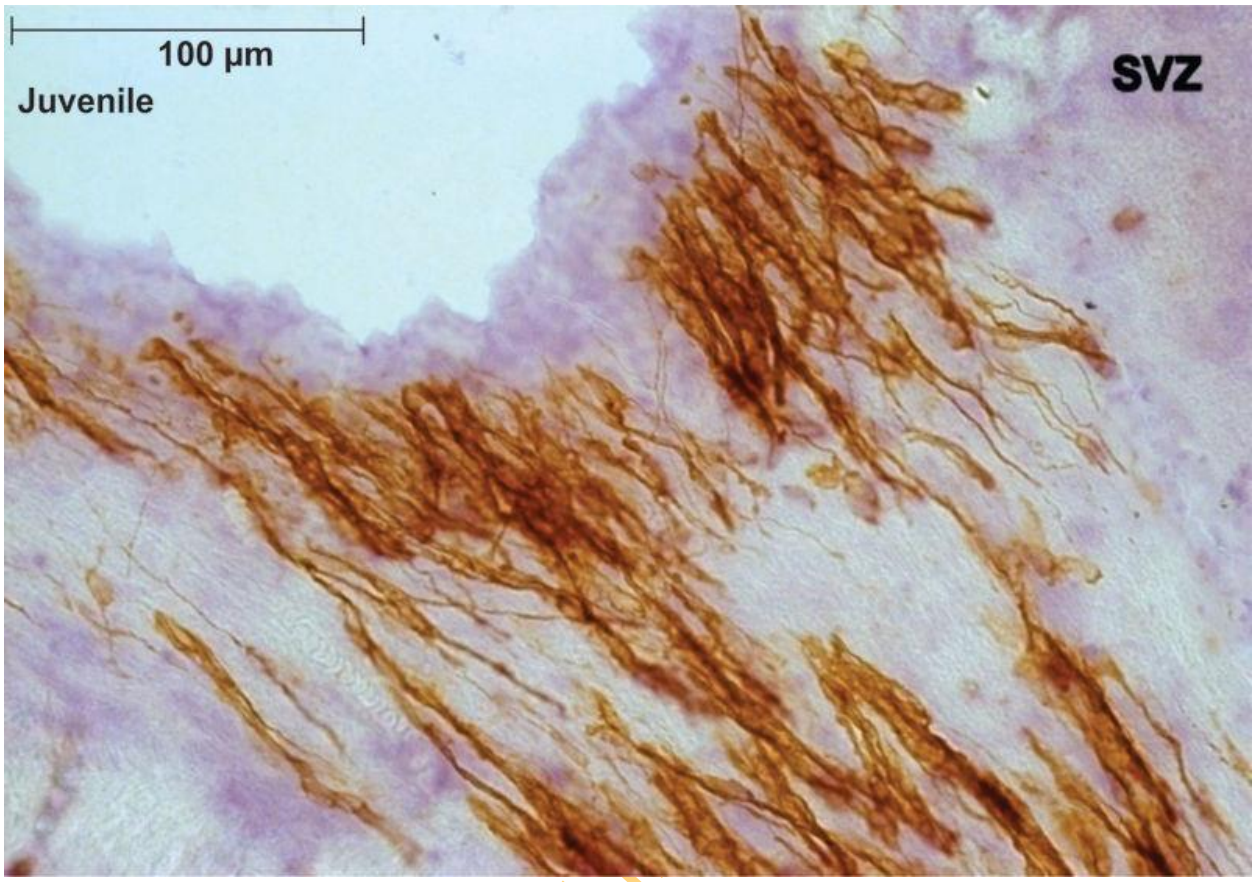


Figure 39: representative photomicrograph showing DCX immunostaining in the SVZ of juveniles, Scale bar-100µm

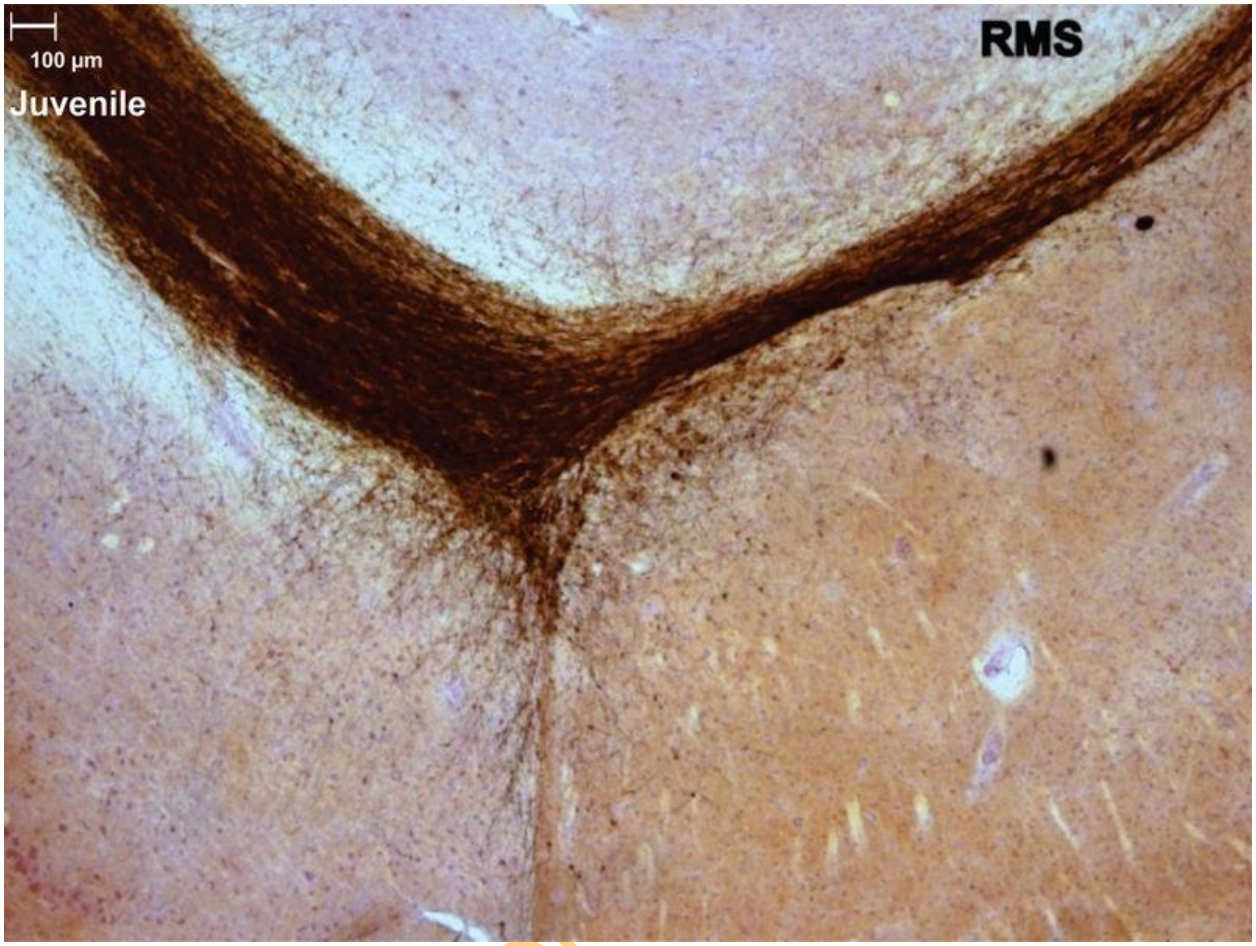


Figure 40: representative photomicrograph showing DCX immunostaining in the RMS of juveniles with numerous migrating neurons identified in this age group. Scale bar-100µm

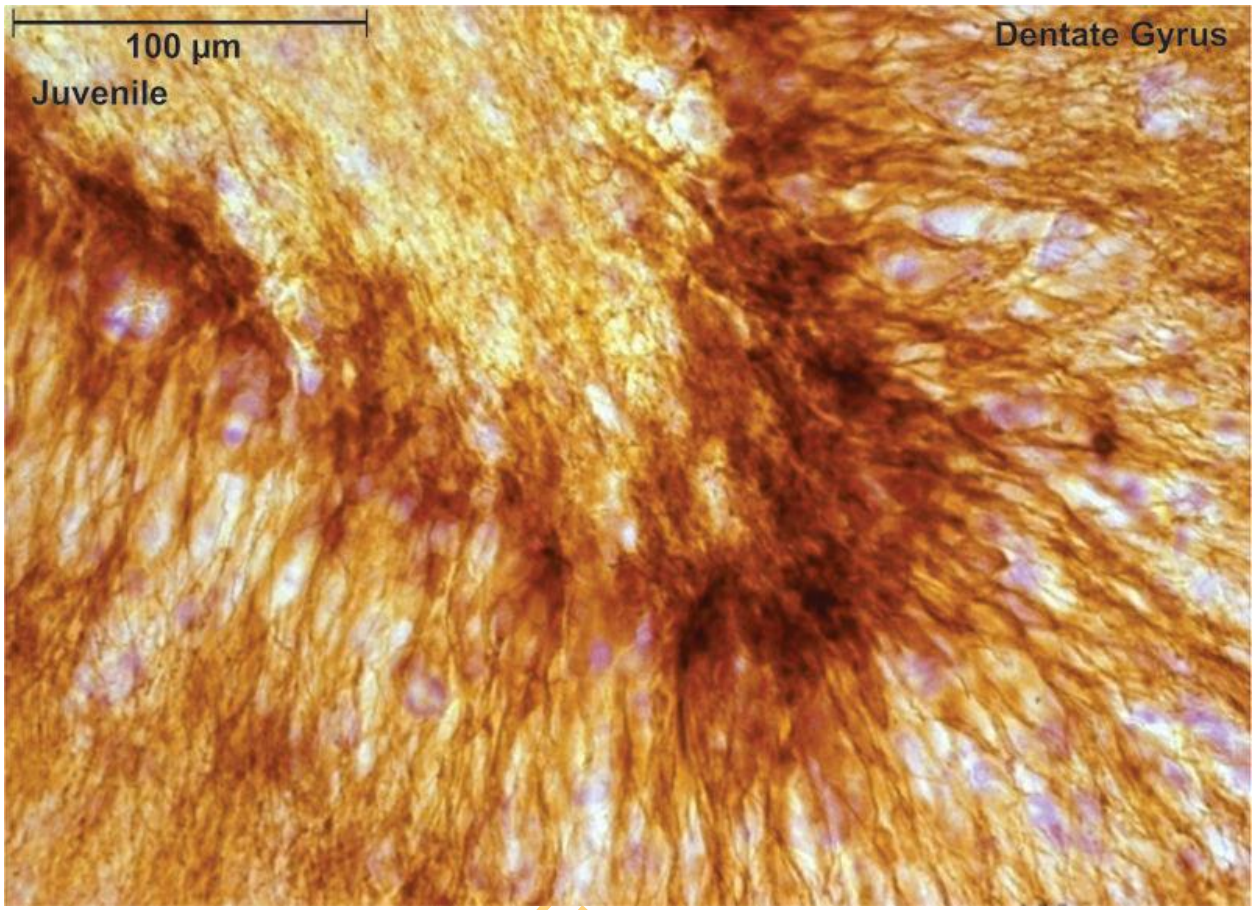


Figure 41: representative photomicrograph showing DCX immunostaining in the dentate gyrus of juveniles. DCX positive cells appear in cluster with their soma and definite processes with the soma obvious in the SGL with their processes projecting as far as into the molecular layer. Scale bar-100µm

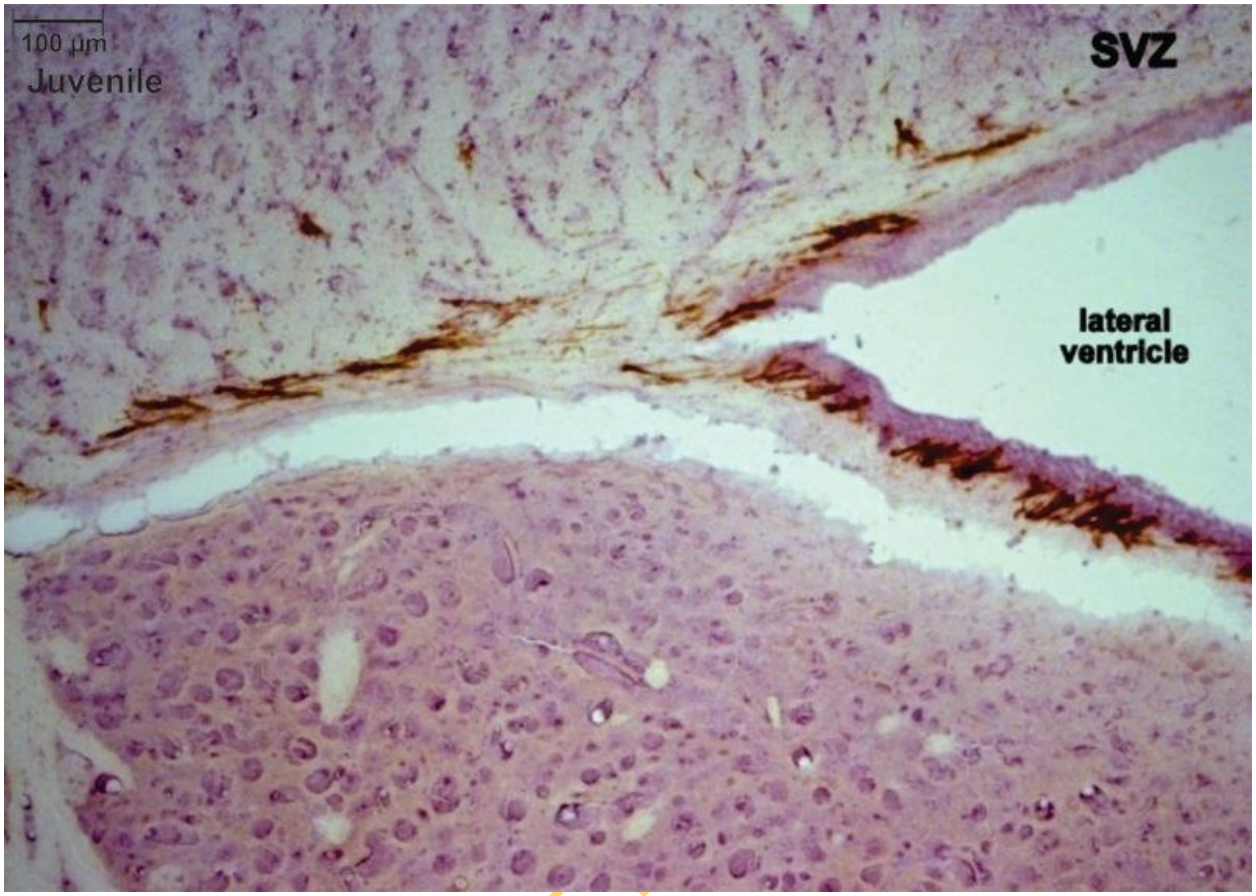


Figure 43: representative photomicrograph showing immature and migratory cells in the SVZ of juveniles, DCX stain, Scale bar-100µm

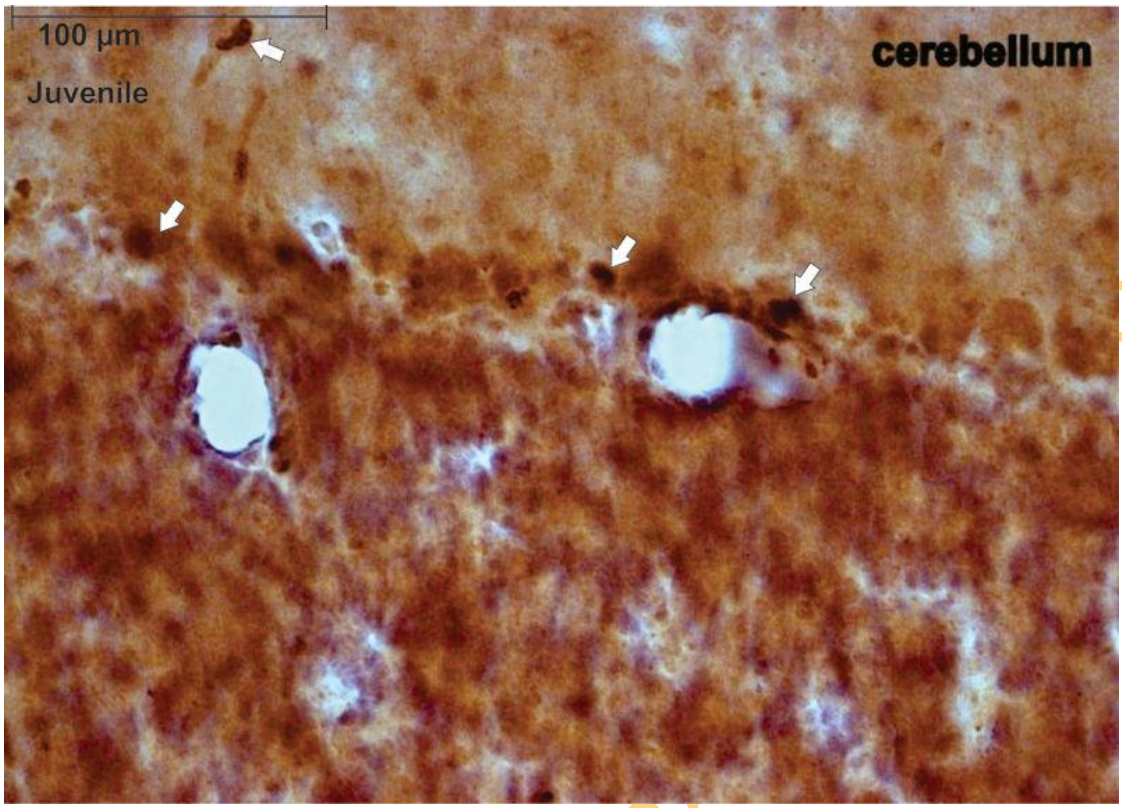


Figure 44: representative photomicrograph showing proliferative immunopositive cells in the purkinje cell layer of the cerebellum of juvenile AGR. Ki-67 stain, Scale bar- 100µm

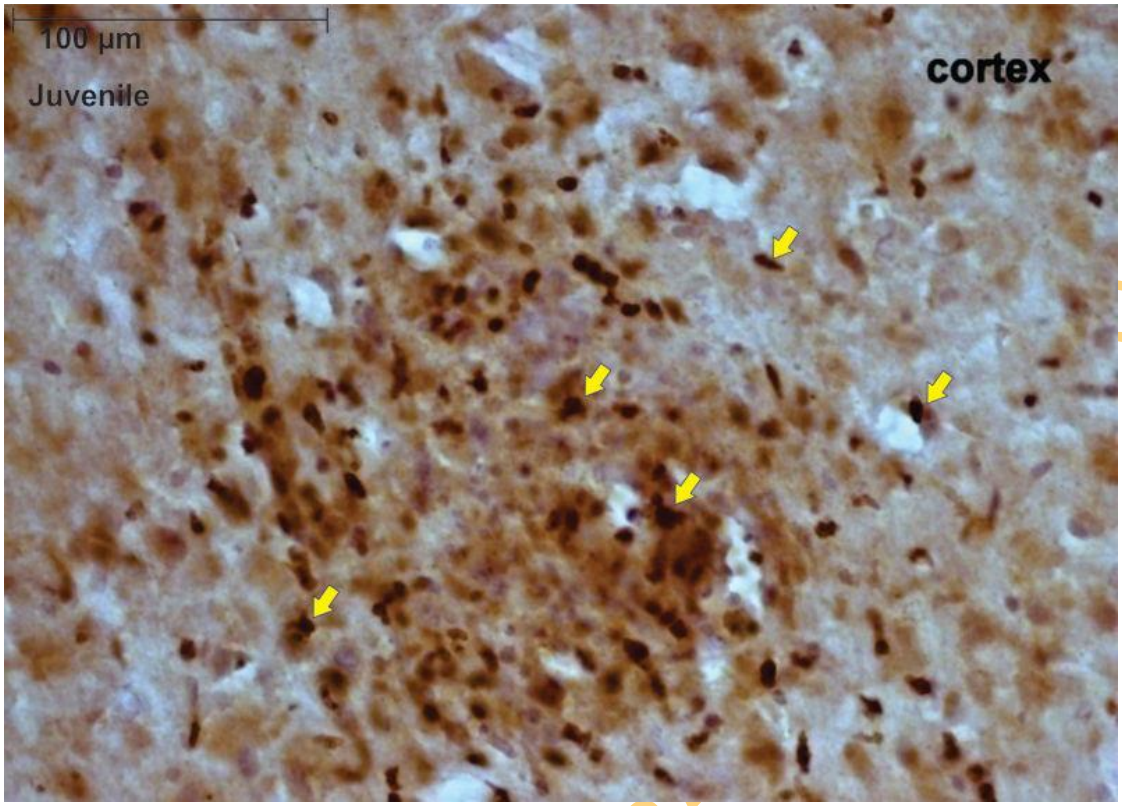


Figure 45: representative photomicrograph showing proliferative immunopositive cells in the cerebral cortex of juvenile AGR, a feature not observed in the adult AGR, Ki-67 Scale bar-100µm

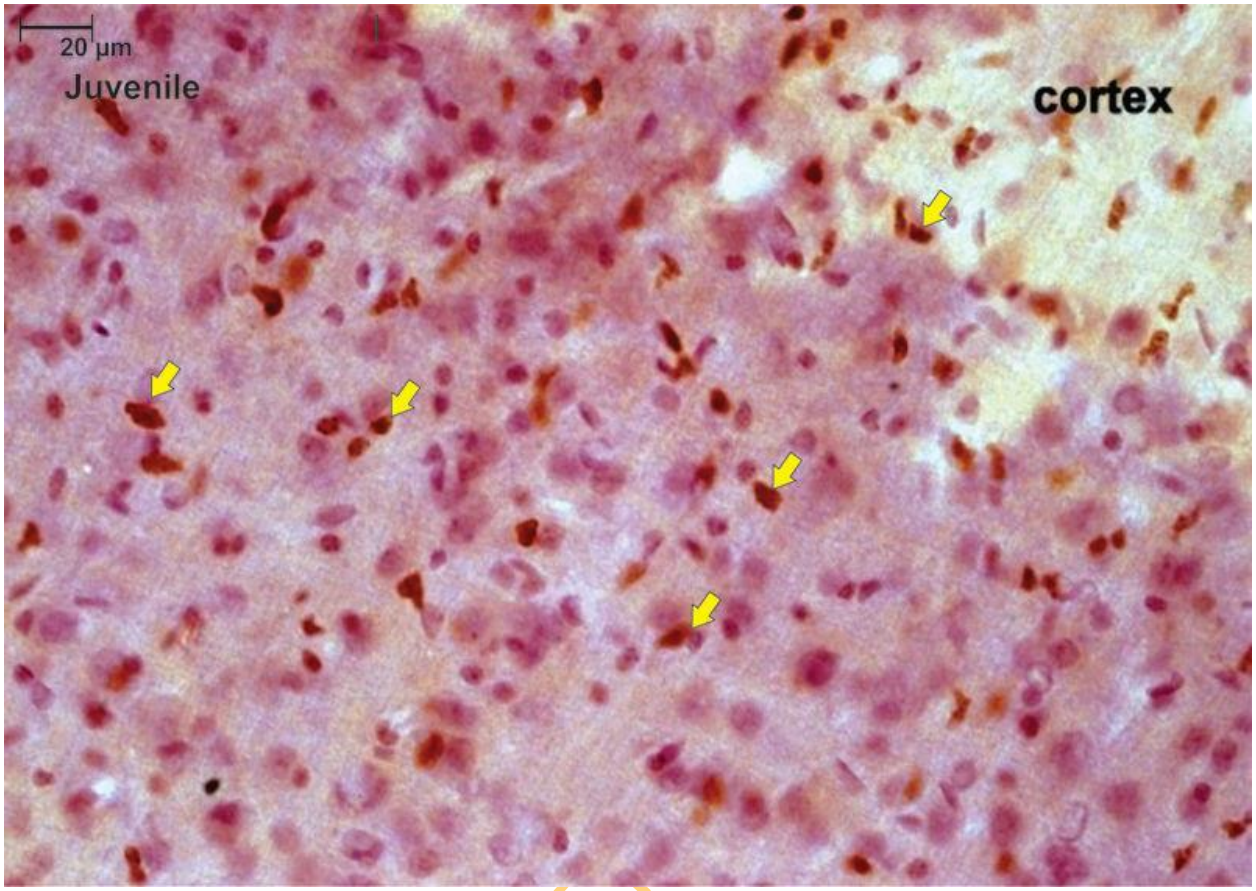


Figure 46: further representative photomicrograph showing proliferative immunopositive cells in the cerebral cortex of juvenile AGR, Ki-67 20µm

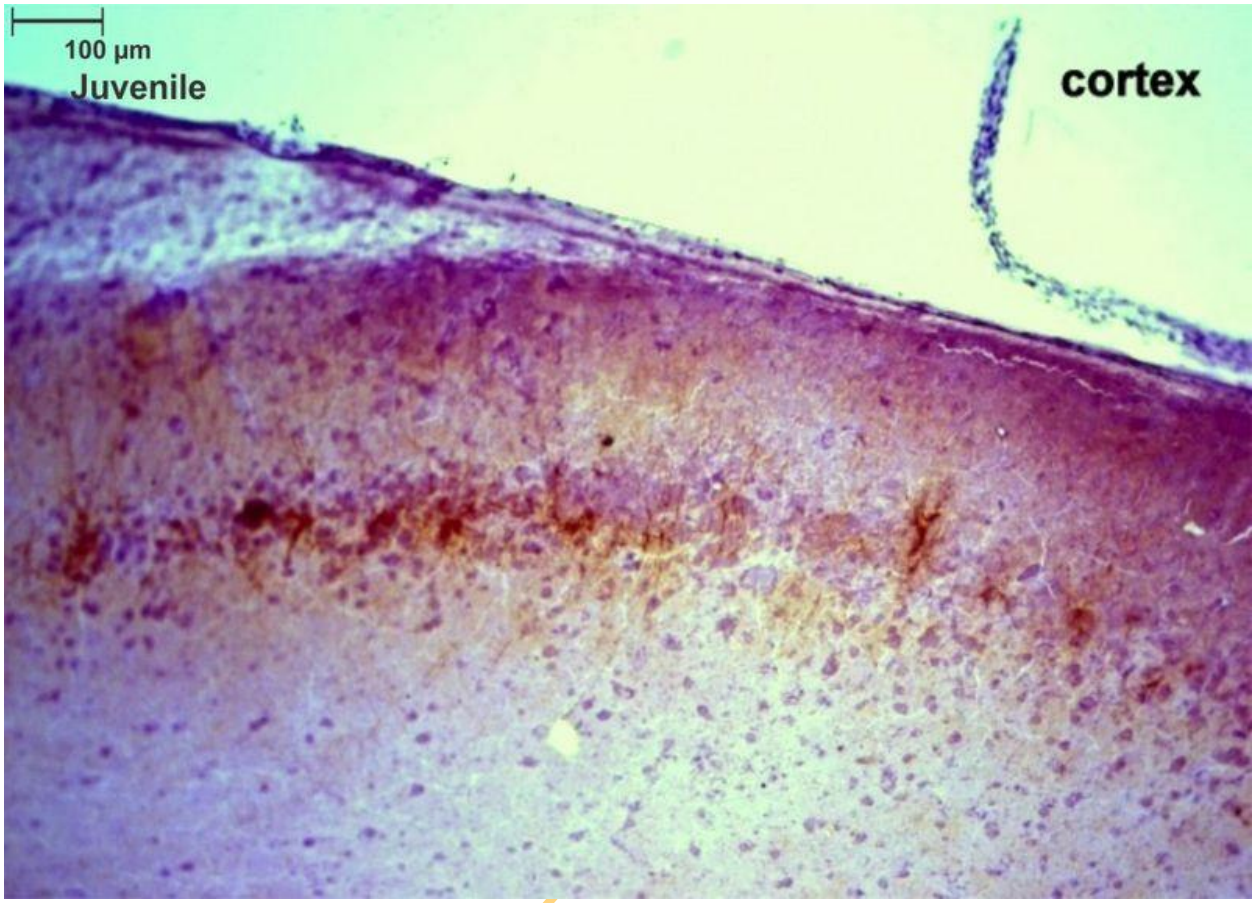


Figure 47: representative photomicrograph showing immunopositive cells in layers II and III of the cerebral cortex of juvenile AGR, DCX 100µm

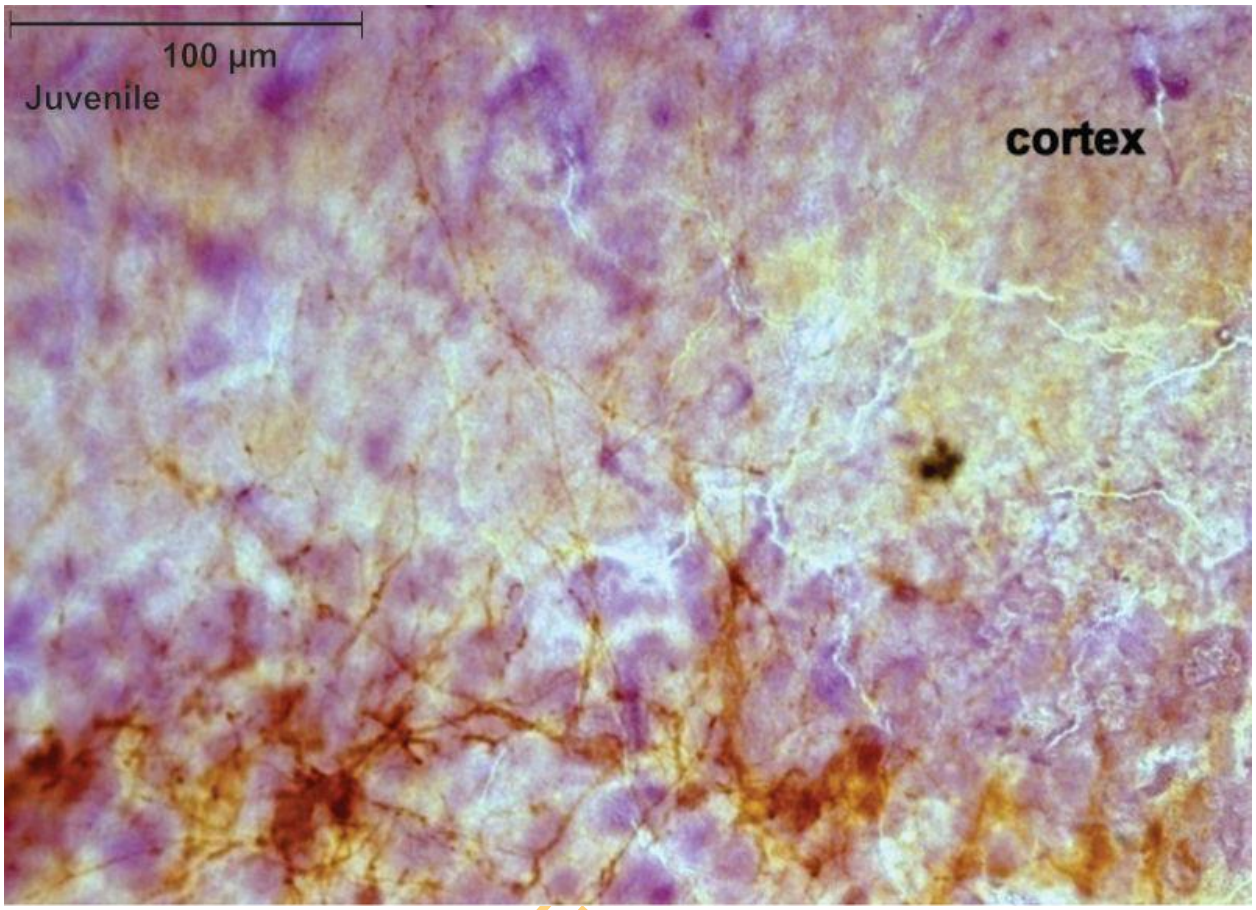


Figure 48: further representative photomicrograph showing enlarged picture of layers II and III in the juvenile cerebral cortex, DCX 100µm

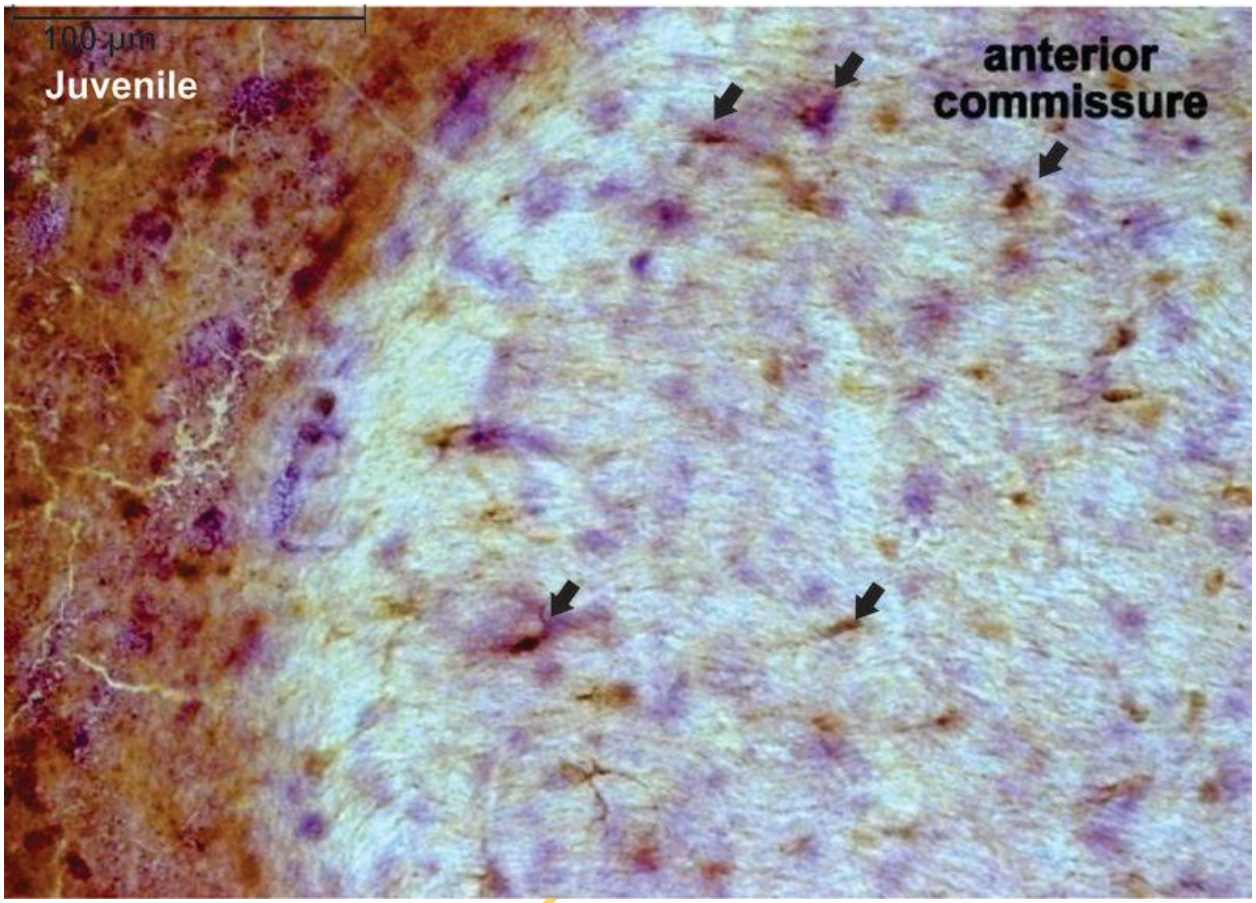


Figure 49: representative photomicrograph showing immunopositive cells in the anterior commissure of juvenile AGR. DCX, 100µm

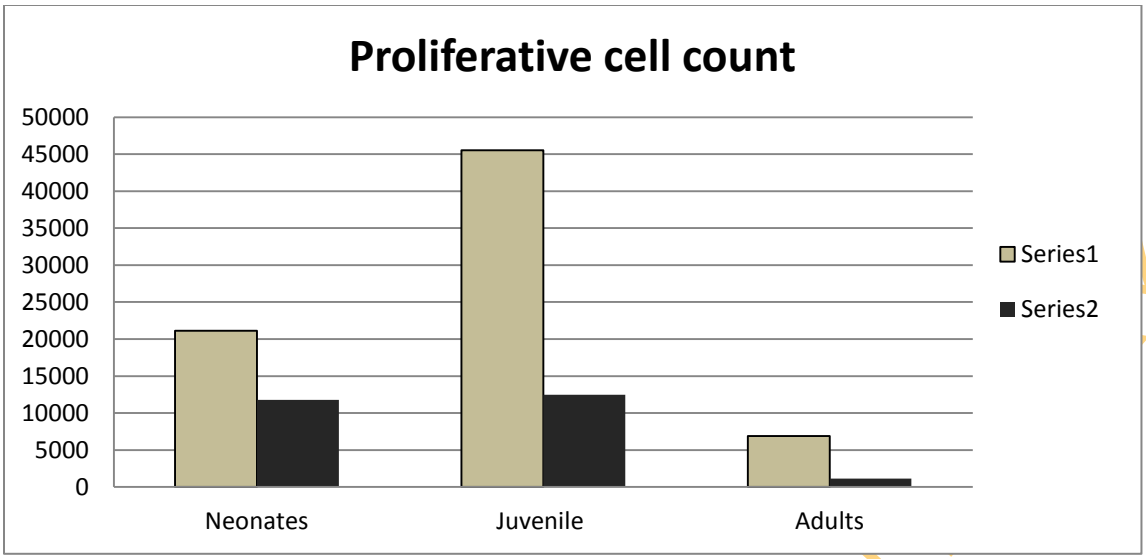


Figure 50: Total Ki-67 proliferative cell count in the subventricular zone and= dentate gyrus of the hippocampal formation of one brain hemisphere across three age groups of African giant rats.

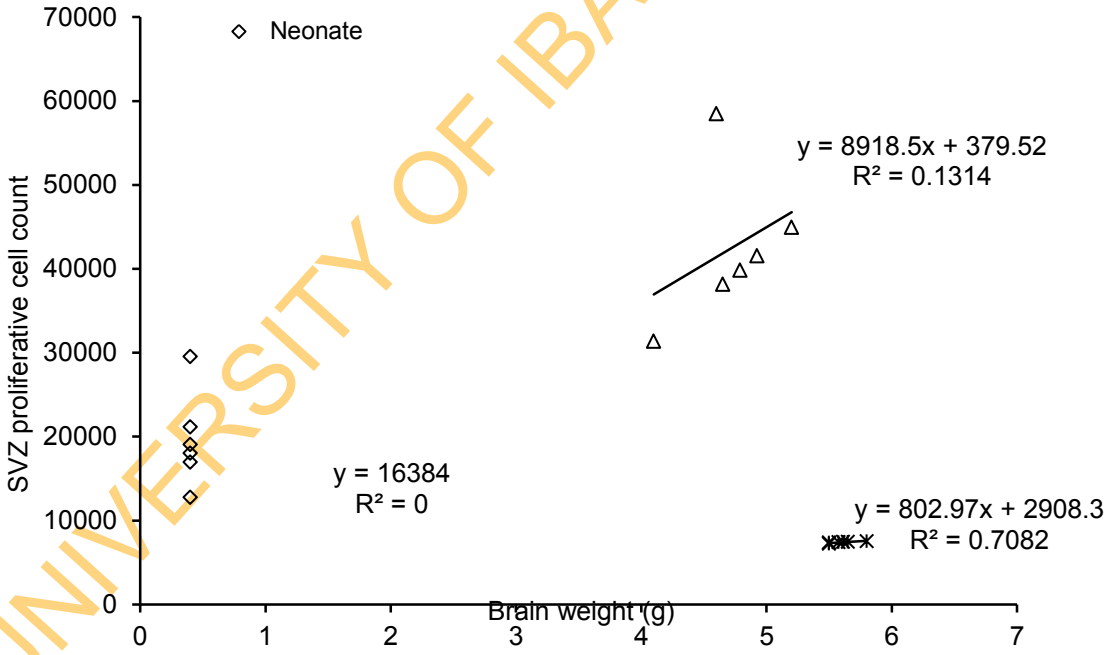


Figure 51

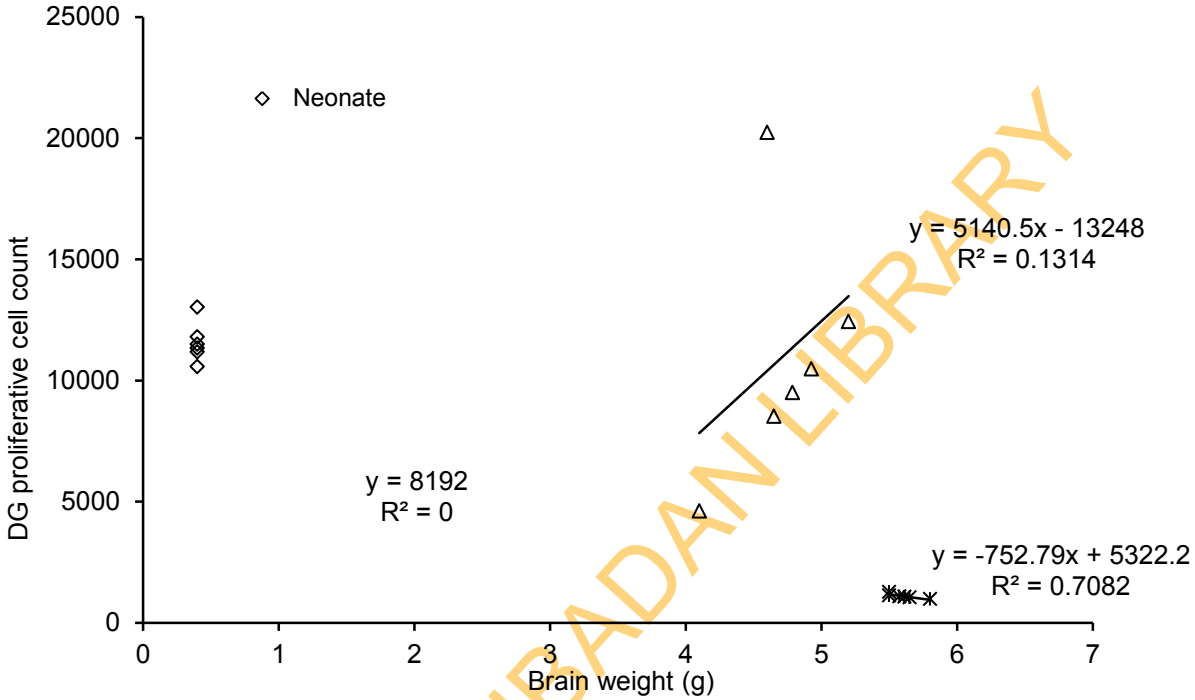


Figure 52

Figure 51-52: Correlation between proliferative cell count in (73) the SVZ (74) the DG and the brain weights of the different groups used in the study. There was no statistical significance except in adults. R^2 shows the strength of the relationship between them.

CHAPTER FIVE

5.0 DISCUSSIONS

OVERVIEW OF ANATOMICAL RESEARCH

A total of seventy one (71) publications were reviewed of which over 50 (about 78%) were authored solely by or in conjunction with Nigerian researchers. Commendably, over 60% were linked directly to authors from the University of Ibadan. The African giant rat is found in various countries of sub Saharan Africa but particular interest of Nigerian authors to the animal can likely be attributed to the early domestication drive by Ajayi in the 1970s. This list is by no means exhaustive considering the methodology which excludes abstracts and unpublished projects, dissertations and theses.

Anatomical articles were largely descriptive, entailing morphometric measurements and often did not reflect research specialization by many authors who wrote on virtually any and all systems. This however, could be attributed to the drive to offer baseline research data in all available areas on the rodent; though certain fields of study have been selectively neglected. More so, morphometry is a recognised tool for scientific study of living animals. In the past, morphological studies were restricted to qualitative description of tissue structures and therefore, they are essentially subjective. In recent years, the applications of morphometric and stereological techniques have increased in biomedical research and have been well recognized as a new approach in morphological studies (Mukerjee and Rajan, 2006).

It was also noted that some research topics were repeated without due credence or citation of initial authors as in digestive system where Knight and Knight had done a comprehensive pilot study on the digestive system which included morphometric and histologic work in the 80s which were not reflected in the articles of Ali *et al.,* (2008) and Nzalak *et al.,* (2010) who actually did portions of the initial study. A likely cause is a lack of in-depth literature review on the subject matter. It is in our view that though over 48% of anatomic publications on the African giant rat were published in the last 14years, there had been several investigations dating back to the 1950s; and to this end, authors must carry out extensive literature search before conducting their research to avoid repetition. Other possible cause of this repetition is the apparent lack of a unified research focus and continuity in certain fields of study which can be directly linked to a dearth in research grants in sub Saharan Africa; inadequate specialized and

advanced equipment for research and the accompanying technical know-how in most local institutions as reflected by the data over fifty years.

There is thus, a need for research focus on Africa's 2nd largest micro-livestock (Asibey and Addo, 2000) and rare rodents (AGR) with policies and funding from government and the private sector because the vast applied potentials of the rodent have started yielding results for science and scientists all over the world with particular usefulness in African settings e.g. in tuberculosis diagnosis, detection of landmines, their small pox carrier status and usefulness in research as models due to their large size and unexplored anatomy.

Anatomy and indeed Veterinary anatomy as a basic clinical science is most useful in the gathering of research data on the form, structure and function of living organisms; and thus, is a very important tool in the study of this unique rodent of Africa to a logical point where its full usefulness can be utilized to the benefit of mankind. In conclusion, it can be said that a dearth of data exists on the AGR in the field of Veterinary anatomy with many areas lacking a single research article on the internet or pubmed. This study should serve as a clarion call to the Nigerian and indeed African governments, private funding organizations, professionals and stakeholders to set up a national, continental or International task force to fully profile the potentials of the AGR and indeed the unique rodents and animals of Africa using the tool of anatomy and other relevant studies to evolve an African rodent model. A model is the work of Olayemi *et al.*, (2012) which involved International collaborators. This will be greatly facilitated by the establishment of African giant rat colonies after the initial, successful but unsustained efforts of Ajayi (1974b). From such colonies, AGR stem cells can be developed alongside various research topics undertaken in understudied areas.

GENERAL GROSS ANATOMY

The gross anatomical structure of the AGR brain was demonstrated in the neonate, juvenile and adult as largely typical to that of the laboratory rat (*R. norvegicus)* as a smooth (lissencephalic) neocortex caudal to the rhinal sulcus which lacked the vestiges of the suprasylvian and marginal sulci (Hebel and Stromberg, 1975; Nzalak, 2005). In the adult brain, the AGR exhibited for the most part, the typical external appearance of the neonate and juvenile subjects, but displayed at least two shallow sulci within the neocortex, one being the lateral sulcus, the other located at the putative caudo-medial border of primary visual cortex. In this

sense, this adult AGR brain did not exhibit the lissencephaly of the neocortex of the smaller brained *R. norvegicus*, neonate and juvenile AGR. According to Dwarika, *et al.*, (2008), the pattern of slight gyrencephally in the caudomedial putative visual similar patterns have been reported in the Armadillo (*Dasypus hybridus*) and greater cane rat (*Thryonomys swinderianus).*

Dura Mater

In the laboratory rats, the cerebral and cerebellar hemispheres have the falx cerebri and tentorium cerebelli respectively as modifications of the dura mater (Waibl, 1973). The dura mater forming the falx cerebri, widely fused with the periosteum, penetrating deeply between the cerebral hemispheres and is known as the falx cerebri pars cerebri (Hebel and Stromberg, 1975). However, in the AGR, the falx cerebri extended in between the lobes of the olfactory bulbs as an uncommon finding. This feature therefore, has been named as the *falx cerebri pars bulbus olfactorius.* The occurrence of the *pars bulbus olfactorius*, made the successful whole removal of the olfactory bulb along with the brain, difficult being always severed from the rest of the brain unless the falx cerebri *pars bubus olfactorius* is first dissected away.

Mean and Body Parameters

Brain weights

The mean brain weight of the AGR (neonate=0.62±0.08gm, juvenile= 4.64±0.17gm, adult=5.60±0.06gm). Though, figures were unavailable for age groups as in this study however, the adult AGR was comparatively higher when compared with that of the adult guinea pig (4gm), sparrow (1gm), but less than the dog (72gm), cat (30gm), porcupine (25 gm), adult greater cane rat (9.80 + 0.50 g for males and 10.27± 0.45 g for females) (Byanet *et al.*, 2009), rabbit (10.00gm) , squirrel (7.6-6.00gm) and marmoset (7.00gm) in the studies of (Nzalak *et al.*, 2005; Eric, 2006).

The ratio of brain to body weight has been reported to be 0.02 for man (Dyce *et al.*, 1996) and 0.006 for the red Sokoto Sheep (Olopade and Onwuka, 2002). In the greater cane rat, the ratios of 0.01 for males and 0.006 for females were recorderd (Byanet *et al.*, 2009), while (Russel, 1979) noted the following Squirrel monkey (1:26=0.04), Marmoset (1:29=0.03), Mouse (1:38=0.03), Squirrel (1:53=0.02), Fox (1:87=0.01), Cat (1:120=0.008), Rat (1:152=0.007), Dog

(1:170=0.006), rabbit (1:300=0.004). In this present study, it was found to be (neonate = 0.03; juvenile = 0.01; adults = 0.006) in the neonate, juvenile and adult AGR respectively.

Russel's hypothesis (Russel, 1979) had stated that there exists a relationship between brain size and intelligence indicating, that brain size reflected intelligence. Based on this, the juvenile African giant rat can be said to be the most intelligent across age groups with the adults showing the least value. In general, the AGR can be said to be more intelligent than many other mammals e.g. the Squirrel monkey, greater cane rats, even the Cat (1:120=0.008), Rat (1:152=0.007), Dog (1:170=0.006), rabbit (1:300=0.004). This hypothesis can however, be flawed based on age variations of recorded values in those animals. This point of view, further adds credence to the fact that, age plays a functional role in intelligence determination especially in the AGR.

The results of this study showed that the olfactory bulb accounts for 20% as against 6% the total brain length in males and 4% in females greater cane rats (Byanet, *et al.*, 2009); the cerebral cortex was 50% (62 % male and 67 % females in greater cane rats) of total brain length while cerebellar cortex was 30% of total brain length.

HIGHER GROSS CEREBRAL CORTEX VALUES IN JUVENILES

The larger cortical volumes, width and antero-posterior dimensions in the cortex of the juvenile AGR were significantly above values for the adults and neonates. This has been reported in the Cat (Zhang *et al.*, 2006) in Humans (Bart Rypma *et al.*, 2001; Blakemore and Choudhury, 2006). Experiments on animals, starting in the 1950s, showed that sensory regions of the brain go through sensitive periods soon after birth, during which time environmental stimulation appears to be crucial for normal brain development and for normal perceptual development to occur (Hubel & Wiesel, 1962). From literature, two main theories are postulated for the changes revealed in the gross dimensions of the brain's cerebral cortex before and after puberty. First, is the concept of progressive myelination of neuronal axons. As neurons develop, a layer of myelin is formed around their extension, or axon, from supporting glial cells. Myelin acts as an insulator and massively increases the speed of transmission (up to 100 fold) of electrical impulses from neuron to neuron. Whereas sensory and motor brain regions become fully myelinated in the first few years of life, although the volume of brain tissue remains stable, axons in the frontal cortex continue to be myelinated well into adolescence (Yakovlev &

Lecours, 1967). The implication of this is that, the transmission speed of neural information in the frontal cortex should increase throughout childhood and adolescence.

The second postulate pertains to changes in synaptic density in the prefrontal cortex. In humans for example, an adult brain has about 100 billion neurons but at birth, the brain has only slightly fewer neurons (Pakkenberg & Gundersen, 1997). However, during development many changes take place in the brain. Neurons grow, which accounts for some of the change, but the wiring, the intricate network of connections or synapses between neurons, sees the most significant change. Early in postnatal development, the brain begins to form new synapses, so that the synaptic density (the number of synapses per unit volume of brain tissue) greatly exceeds adult levels. This process of synaptic proliferation, called synaptogenesis, lasts up to several months, depending on the species of animal and brain region. At this point, synaptic densities in most brain regions are at their maximum. These early peaks in synaptic density are followed by a period of synaptic elimination (or pruning) in which frequently used connections are strengthened and infrequently used connections are eliminated.

This experience-dependent process, which occurs over a period of years, reduces the overall synaptic density to adult levels. These data came mainly from studies of sensory regions of animal brains. The first demonstration of synaptogenesis was when it was found that in the cat visual system the number of synapses per neuron first increases rapidly and then gradually decreases to mature levels (Wong, 2002).

Further research carried out in rhesus monkeys (Rakic, 1995) demonstrated that synaptic densities reach maximal levels two to four months after birth, after which time pruning begins. Synaptic densities gradually decline to adult levels at around 3 years, around the time monkeys reach sexual maturity. Histological studies of monkey and human prefrontal cortex have shown that there is a proliferation of synapses in the subgranular layers of the prefrontal cortex during childhood and again at puberty, followed by a plateau phase and a subsequent elimination and reorganisation of prefrontal synaptic connections after puberty (Zecevic & Rakic, 2001).
According to these data, synaptic pruning occurs throughout adolescence and results in a net decrease in synaptic density in the frontal lobes in the adult life.

The juvenile AGR had higher cortical volume, width and A-P dimensions than the adults and these postulates can best describe the size reduction observed. However, effect on cognitive implications of this second wave of synaptogenesis in the frontal cortex at the onset of puberty and the process of synaptic pruning that follows it after puberty is believed to be essential for the fine-tuning of functional networks of brain tissue, rendering the remaining synaptic circuits more efficient and to underlie sound categorization (Zhang *et al.*, 2006).

Closely related to the above hypothesis are findings on adult neurogenesis studies on the AGR (Olude *et al.*, 2014b). It has been observed that post natal induction of neuronal plasticity leads to reorganization of microtubules, for example during synaptic reorganization or axonal outgrowth (Nacher *et al.*, 2001). This hypothesis implies that the neocortex with its various cortical divisions e.g. the piriform cortex is a very plastic area, and therefore adult neurogenesis could be one of the mechanisms of plastic changes affecting synaptic density.

OLFACTORY BULB DIMENSIONS IN THE AGR LOB

Nzalak *et al.*,(2005), measured the overall length of the olfactory bulbs, described the olfactory bulbs of the AGR as oval ventrorostral projections from the cerebrum by the rhinal sulcus, lying in the cribirifom fossa of the ethmoid bone. In this present study, the measurements were done on each bulb and the left Olfactory bulb and found to be significantly longer than the right. The juveniles also had larger bulbs than the adults. As earlier discussed, the synaptogenesis and neuronal trimming with progressing age might have similar effects on the olfactory bulbs and possibly on olfactory differentiation across age groups.

Results for adult neurogenesis in the brain of the AGR show that these could belong to post natal neurons of either OB or the piriform cortex, or both, as both (Fox *et al.*, 1948; Patzke *et al.*, 2013), showing that newly generated neurons are able to send long-range projections into the adult brain. The piriform cortex receives direct input from the OB (Heimer 1968; Scott *et al.*, 1980) and in all examined species of mammals; the OB shows the highest rate of incorporation and turnover of the new neurons throughout life. Indicating a constant exchange of neurons takes place, allowing for an extraordinary plasticity of the whole olfactory system accompanied by age related synaptic density and consequent trimming in the AGR (Olude *et al.*, 2014). Most of the positive DCX cells are believed to migrate to the olfactory bulb and incorporate there (Bartkowska *et al.*, 2010).

Some may deviate from this track towards the frontal cortex between layers II and III (Biebl *et al.,* 2000). DCX and Ki-67 confirmation of adult neurogenesis in the olfactory bulb of the AGR has been observed in all the layers of the AGR main OB (Olude *et al.,* 2014). Immunoreactive cells observed had bipolar cells with radially orientated dendrites. Short tangentially orientated intensely DCX immunoreactive fibers were observed in the PVL which extended towards the RMS with soma and bipolar dendrites. In this study of Olude *et al.,* 2014, it was showed for the first time the presence of newly generated cells in brains of neonate, juvenile and adult AGR.

The neuronal quantitification of adult neurogenesis in this species which was was determined in the SVZ and DG clearly showed that the rate of neurogenesis was higher in juveniles and lowest in adults; a pattern that has been reported to depend on strain and age of rats and mice (Kempermann *et al.,* 1997) with the AGR showing increase in adult neurogenesis from neonates to adults. 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; Grabiec *et al.,* 2009). This, related to the synaptic density and trimming hypothesis, may be responsible for varying brain neocortical dimensions which were higher in juvenile subjects as compared to adults; a pointer to a higher level of plasticity in the AGR and juveniles in particular and the hypothesis that juveniles have the best learning abilities in this species

CEREBELLAR VALUES

The External Granular Layer (EGL)

The external granular layer is the most metabollicaly active part of the developing cerebellum as the differentiation of the cells of this layer gives rise to the outer stellate, basket, granule and golgi type II cells of the cerebellar cortex. This differentiation process requires energy. In this study the EGL seemed to persist into juvenile life but in the wistar rats, it has been noted to disappear by post natal day 21 (Imosemi, 2011; Jacobson 1991b) stated that the EGL disappears by post natal day20 in mice. The persistent EGL in the juvenile suggests delayed maturation of the cells. The mechanism for the delay is not completely clear but, intrauterine hypoxia or ischemia, embryo fetal toxicity and vascular necrosis of neurotoxic substances are well documented (West *et al.,* 1996). Also, this might be a normal occurrence in wild AGR as no

reports exist on the cerebellar development in this species. Particularly so as other cellular components were at this time well developed inspite of the persistent EGL.

The Molecular Layer (ML) is situated between the EGL and PL. it typically becomes the most superficial layer after complete disappearance of the EGL. The ML is packed with dendrites and axons but has relatively few neurons. Only 2 of the neuron types (basket and outer stellate) have their cell bodies in the ML (West 1995). The thickness of the ML is therefore determined by the amount of cells and fibres present (Rakic and Sidman, 1973) but majorly by accretion of new parallel fibres. In this study, the juvenile AGR had higher ML thickness than the adults. It has been reported that reduction in the ML thickness may be due to reduction of GL thickness because the granule cells extend their axons to the ML where they form parallel fibres and synapse with the dendrites of the outer stellate, basket and Purkinje cells in the Wistar rat exposed to neurotoxic phenytoin (Imosemi, 2011). As such, reduction of GL thickness may lead to depletion of the axons in the ML resulting in reduced ML (Imosemi, 2011). However, in this study, adult AGR had greater thickness in the GL above juvenile AGR therefore being inconsistent with the earlier statement.

Numerous studies have documented the relationship between morphological change and functional decline in the central nervous system (CNS). The loss of cortical thickness and neurons is viewed as the main cause of decline in brain function during ageing. With increasing ageing in the AGR, we observed a significant decrease in thickness of the molecular layer which ultimately led to a decrease in total cortical thickness with increasing age. This follows the report of (Zhang *et al.,* 2006) in the cat who stated that the loss of thickness in the molecular layer was caused largely by the progressive defoliation of dendrites in the ageing Purkinje cells (Hadj-Sahraoui *et al.,* 2001), and that a slight increase in the thickness of the granular layer was mainly due to proliferation of the astrocytes.

The exact functional relevance of the decrease in cortical thickness is not clear, but a reduction in the number of neurons leading to global motor behavior impairment has been largely proved (Hilber and Caston 2001; Caston *et al.,* 2003). A statistically significant decrease in thickness of the molecular layer as shown in the adult AGR cerebellum can therefore be said to be indicative of neuronal loss. The neurons of the molecular layer (basket cells and stellate cells), make inhibitory synapses with the Purkinje cells, while the axons of the granular cells bifurcate into parallel fibres, which send major excitatory inputs to the Purkinje cells. The loss of

granular cells may therefore, directly lessen the excitatory inputs to the Purkinje cells in aged animals. Also, the granular cells (along with their parallel fibres) are related to the storage of memories over different time-periods, regulate the dynamics of movement and allow bidirectional changes in movement amplitude (Attwell *et al.,* 2002; Boyden *et al.,* 2004), their loss may greatly affect motor learning in ageing individuals. The presence of age-related changes in the granular cells is disputed; some researchers reported no age-related changes in aged *Macaca nemestrina* (Nandy 1981) and rat (Bakalian *et al.,* 1991), while other authors found significant changes in aged rats (Amenta *et al.,* 1991) and humans (Renovell *et al.,* 1996). However, Andersen *et al.,* (2003) reported that both these phenomena were observed in the ageing human cerebellum within different zones.

Purkinje cells PC are the sole efferent neurons in the cerebellar cortex that are prone to ageing. It therefore stands to reason from this study and other studies (Nandy 1981; Hara *et al.,* 1992) that loss of such cells may weaken the inhibitory stimuli to the Purkinje cells (Zhang *et al.,* 2006) resulting in a significant reduction in number of Purkinje cells in ageing animals. Loss of neurons is not the only mechanism by which age may affect the cerebellum. A reduction in the dendritic branches of Purkinje cells may also interfere with information exchange in spite of the presence of surviving cell bodies. Purkinje cells receive synaptic inputs mainly from parallel fibres through their dendritic network and send projections to the deep cerebellar and vestibular nuclei via their long axons. The loss of arborization in the Purkinje cells could directly cut down the amount of information input, reduce afferent efficacy, affect information integration and signal transmission in the aged cerebellum.

These changes would lead to a reduction in motor control and accurate motor coordination (Hilber and Caston 2001; Hue *et al.,* 2004; Porras-Garcia *et al.,* 2005). Purkinje cell dimensions were significantly reduced with ageing in the AGR. This result in the AGR is in agreement with Zhang *et al.,* (2006) and Hadj-Sahraoui *et al.,* (2001) that the dendritic arborizations of Purkinje cells are obviously atrophying in ageing AGR.

Age-related changes in GFAP immunoreaction

GFAP is a special marker for astrocytes. It has been reported that the number and size of GFAP-positive astrocytes in the CNS increases following certain types of brain injury and in various pathological conditions (Davis *et al.,* 2002; German *et al.,* 2002; Sulkowski *et al.,* 2002).

GFAP astrocytes are also sensitive to ageing and display age-related proliferation, which has been investigated in many mammalian species (Jalenques *et al.*, 1997; Sabbatini *et al.*, 1999; Wu *et al.*, 2005). The cerebellar cortex has a particular glial architecture, with large astrocytes located in the granular layer. Age related changes in the astrocytes of the cerebellar cortex have been reported only in the rat (Sabbatini *et al.*, 1999).

The findings of our study provide evidence that ageing is also accompanied by changes in the astrocytes of the AGR cerebellar cortex, including an increase in their size and number, and intensity of immunoreactivity. The finding that there is an obvious loss of neurons in the ageing cerebellar cortex suggests that proliferation of the astrocytes might be triggered by the neurodegenerative changes during ageing. The significance of age-related changes in the expression of GFAP in the astrocytes is not clear. Gliosis may be a reaction to intrinsic modifications in the CNS such as degeneration of neighbouring dendrites or entire neurons (Geinisman *et al.*, 1978; Niquet *et al.*, 1996), and may be an attempt to promote long-term neuronal survival during ageing through the production of neurotrophic factors (Schmalenbach and Muller 1993). Since trophic interactions exist between astrocytes and neurons (Araque *et al.*, 2001; Kirchhoff *et al.*, 2001), it is suggested that the hypertrophy and hyperplasia of the astrocytes may slow down the dying process of ageing neurons and the degeneration in the cerebellar cortex and this also suggests that this may be a response to offset the decrease in cortical thickness and fill up the gap caused by the loss of neurons.

In summary, the cerebellar cortex undergoes age-related structural changes. These findings in the AGR confirm the results in other species e.g. the Cat, indicating a common phenomenon for age-related structural changes in the mammalian cerebellar cortex. The loss of cortical neurons and the reduction in dendritic arborizations of the Purkinje cells might be the main reason for the functional decline in motor control and motor learning in senescent animals. The proliferation of astrocytes may exert a protective effect on neurons thereby accounting for the increased granular layer thickness and offset the decrease in cortical thickness of the aged cerebellum.

CEREBRAL CORTEX

Cerebral neurons

The study provided a morphological characterization of different neuronal types in the AGR neocortex. Cell types in the neocortex were found to include spiny and smooth or sparsely spiny neurons. In primates, the predominant (70–90%) type of spiny neuron is the pyramidal cell, which is typically defined by a triangular-shaped cell body, a wide "skirt" of basilar dendrites, one axon descending into the underlying white matter, and one single apical dendrite ascending vertically toward the pial surface (Bianchi *et al.*, 2011). However, as more comparative studies accumulate, it has become apparent that such a structure constitutes one end of a broad scope of morphologies, spanning from "typical" pyramidal to stellate neurons (Bianchi *et al.*,., 2011). Descriptions in the neuromorphology in the African elephant (*Loxodonta africana*) neocortex (Welker *et al.*, 1976; Jacobs *et al.*, 2011) confirm this with a remarkable heterogeneity of spiny neurons from those appearing more pyramid-like (e.g., magnopyramidal, multiapical, and fork neurons), to those radically differing from the "typical" pyramidal shape (e.g. horizontal and inverted pyramidal neurons; "crab-like" and flattened pyramidal neurons). In the rock hyrax (*Procavia capensis*), a spectrum of spiny neuron morphologies are observed with fewer separate neuronal types identified, including only pyramidal, forked shaped, inverted pyramidal, and bitufted neurons. Of the pyramidal-like neurons, some presented "atypical" features (e.g. bifurcating apical dendrites, multiapical and inverted soma) that have been similarly observed in other Atlantogenata, such as elephant shrews, anteaters, and sloths, as well as a monotreme, the short-beaked echidna (Bianchi *et al.*, 2011).

The AGR nevertheless, was characterized by a predominance of "typical" pyramidal cells that ascended vertically toward the pial surface. This is similar to the pattern in the African rock Hyrax and in contrast to the elephant that had most spiny neurons with apical dendrites that bifurcated at or near the soma, resulting in two obliquely ascending secondary branches that joined with others to form V-shaped apical bundles (Bianchi *et al.*, 2011). While being, the widely bifurcating structure observed in the elephant is consistent with descriptions in the horse and the cow, as well as the two-toed sloth and anteater but represents a striking departure from the vertically orientated apical dendrites that typify the rodent and primate neocortex. Together with previous observations, this suggests that the notion of a neocortical architecture common to all mammals, defined by pyramidal-shaped projection neurons with apical dendrites bundled

together at the core of minicolumns, may not capture the actual variation that is present among different mammalian lineages.

In terms of morphology and laminar distribution, these neurons resembled those described in monotremes, humans, carnivores, artiodactyls, lagomorphs, rodents, dolphins, and elephants. Because aspiny cortical neurons generally correspond to inhibitory GABAergic subtypes, this suggests that there may be relatively more evolutionary conservation in regard to the morphology of cell types that comprise the intrinsic microcircuitry of the mammalian cerebral cortex (Bianchi *et al.*, 2011).

Considerable phylogenetic diversity in the density and biochemical phenotype of these inhibitory interneurons, however, is apparent. Spine density and dendritic branching patterns are essential in determining the receptive field and integrative capacity of the neuron. In primates, regional variation in neuronal morphology is thought to reflect area-specific functional specializations. Specifically, neurons with greater dendritic complexity and higher spine density might subserve the synthesis of a more diverse array of inputs. Thus, the higher spine density found in the frontal cortex of the AGR provides indirect evidence that this region is involved in functions that entail greater computational demand. However, given the scarcity of data on the physiology and connectivity of the AGR cerebral cortex, it is uncertain whether the functional significance of regional differences in spine density is comparable to that of primates. The present study contributes, however, to the growing body of research delineating neuromorphological variation in the neocortex of AGR.

EPENDYMA

Ependyma has been the subject of numerous investigations. However, much structural and functlonal informations concerning them is lacking (El-Basouny and Hindawy, 2006). This describes the AGR which is totally lacking in research along these lines.

It has been generally accepted that the lining of the cerebral ventricles and central canal of the spinal cord is similar to most epithelial membranes in both form and construction (Agduhr, 1932; Wislocki, 1932). The characters of this epithelial lining was first documented by Purkinjc 1836, and investigators subsequently recognized that the ependyma was of heterogeneous composition, in particular that some ependymocytes had basal processes that extended into the subjacent neuropil.

Ependymal cells (ependymocytes) form the epithelial lining of the ventricles and spinal canal. Cuboidal or low coloumnal in shape, the cells are tightly bound together at their luminal surfaces by the usual epithelial junctional complexes. Unlike other epithelia, however ependymal cells do not rest on a basement membrane but, rather, the bases of the cells taper and then break up into fine branches which ramify into an underlying layer of processes derived from astrocytes. At the luminal surface, there is a variable number of cilia. Microvilli are also present and probably have absorptive and secretory functions. Typically in rodents, the ependymocytes are only a layer thick (El-Basouny and Hindawy, 2006). They also described stratified ependemocytes as subtypes of ependyma in rats using electron microscopy. In 1954, Horstmann, first applied the descriptive term tanycytes to such elongated ependymal cells seen in selachains with gold sublimate staining. Particular interest in tanycytes originated with the observation that, within a circumscribed region of the adult mammalian third ventricle, they established an anatomical link between cerebrospinal fluid and the hypophysial portal vasculature.

In the AGR, especially in juveniles and adults, stratified ependymocytes were typically observed as cuboidal-low columnar cells along the ventricular lining. The thickness varied at different regions only by 1-2 cell layers and was characteristically higher in juveniles than in adults. Neonates showed the typical one layered ependyma cells.

Reports say that ependymal cells in the lesioned spinal cords of rats proliferated in response to injury (Noorulla and Rao, 2013). Though the AGR used in this study were wild caught, the possibility of wide scale infection is highly ruled out as the stratified layering was a uniform finding and all animals were not subjected to same environmental conditions. Possible explanation to this is that, the ependyma retains regenerative capacity. They have also concluded that this proliferation of ependyma may replace the lost neuronal tissue and help in axonal regeneration (Attar *et al.*, 2005). It has been indicated that the ependymal cells are themselves multipotent and can divide and proliferate according to the severity of the injury (Takahashi *et al.*, 2003). Vaguero *et al.*, (1981) and Vallace *et al.*, (1983) observed the regenerative activity of ependyma following compression injury.

Bruni *et al.*, 1985, observed that the ependymal turn over declines significantly in normal adults post natally and only residual activity persisted in adulthood. In the infra mammalian vertebrates, the ependyma plays a significant role in the regenerative processes in spinal cord. In hydrocephaly also there had been a similar response though it could be a part of overall tissue

response (Bruni *et al.*, 1985). In response to various disruptive factors like injury, compression etc. the normally mature ependyma may revert to a primitive state and exhibit proliferative activity in comparison with the abnormal neuroepithelium (Matthews *et al.*, 1979) In the lower vertebrates ependyma plays a very important role in initiation and maintenance of regenerative process (Bryant and Wozney, 1974)

ASTROCYTES

Astrocytes display considerable, age specific, region-specific differences in morphology and density across the rostro-caudal extent of the AGR brain reflecting the classical, neuronally-defined post natal anatomy of the mammalian CNS. Although, it is reported no single reporter system or immunolabel highlights every astroglial cell in the CNS GFAP was utilized to demonstrate morphological investigations of astroglial heterogeneity in the AGR (Emsley and Macklis, 2006),. Age group studies offered the spectrum of complete and distinct morphology of astroglia during post natal development. GFAP thus, remains an efficient way to characterize the substantial diversity of astroglial morphology, and density particularly in the juvenile and adult groups of the AGR.

Considerable heterogeneity of astroglia has been described with respect to development, proliferation, morphology, location, protein expression and function of astrocytes (Prochiantz and Mallat, 1988; D'Ambrosio *et al.*, 1998; Wilkin *et al.*, 1990; Zhang, 2001; Bushong *et al.*, 2002; Ogata and Kosaka, 2002; Scotti Campos, 2003; Kimelberg, 2004; Wallraff *et al.*, 2004).

The existence of these striking regional and sub-regional variations indicates that this immense diversity influences neural function in important, region-specific ways. Pathophysiological questions have also been raised whether particular astroglial subtypes in distinct CNS regions are more likely to develop into glial tumors. Recent reports (Bruijn *et al.*, 2004; Pehar *et al.*, 2004; Cassina *et al.*, 2005) suggest that future work should also assess whether regional differences in populations of astroglia influences the degree of susceptibility of CNS sub-regions to neuronal degeneration. (Wang *et al.*, 2011) showed that Adenomatous Polyposis coli (APC) a multifunctional protein that inhibits Wnt/β Catenin signaling pathway and regulates the microtubule and actin cytoskeletons plays a critical role in maintain morphology and function of cerebellar Bergmann glia in rats. Accumulation of unregulated β Catenin cause their radial fibres to become shortened with a marked reduction in branching

collaterals and their cell bodies translocated into the molecular layer followed by loss of their pial contact and transformation into stellate shaped cells. Significant loss in Purkinje neurons and cerebellar atrophy also set in by middle age (Wang *et al.,* 2011). Outside the cerebellum, neither beta catenin accumulation nor morphological changes were identified in the GFAP expressing astrocytes, indicating region specific effects of APC deletion and an essential role for APC in maintaining the unique morphology of Bergman glia as compared with other astrocytes. Thus, providing a possible mechanism for region-specific non-cell autonomous neurodegeneration.

What induces and maintains the regional differences among astroglia is largely unknown. Given the complex, crucial interactions between neurons and glia in the developing and adult CNS, it is likely that these interactions themselves influence astroglial morphology, density and proliferation.

The existence of such broad variations in morphology, expression and density highlights the possibility that the developing neuronal populations influence the characteristics of astroglia. It is however, speculated that the developing neuronal environment influences the lineage progression of astroglial subtypes in order to guarantee that the most effective glial support network is available for the function and long-term survival of that particular neuronal population (Emsley and Macklis, 2006).

In addition to possible neuronal influences on glia, the local microenvironment can direct and maintain the morphology and heterogeneity of astroglia (Reichenbach and Wolburg, 2005). Together, data on the expression, morphology and density of astroglia in the AGR reinforce the existence of remarkable regional heterogeneity of astroglia in the post natal mammalian CNS.

Expression of glial Fibrillary acid protein (GFAP) has become a prototypical marker for immunohistochemical identification of astrocytes. Nevertheless, it is important to recognize the appropriate uses and limitations of GFAP as an astrocyte marker (Sofroniew *et al.*, 2010). GFAP has been studied extensively as one of a family of intermediate filament proteins, including vimentin, nestin, and others, that serve largely cytoarchitectural functions. Some authors argue however, that GFAP is not an absolute marker of all non-reactive astrocytes and is often not immunohistochemically detectable in astrocytes in healthy CNS tissue or remote from CNS lesions. In this study nonetheless, anti-GFAP immunohistochemistry was utilized to map the morphology, distribution and densities of Astrocytes in theAGR brain across three broad age groups (neonates, juveniles and adults).It revealed an accumulation of GFAP during early

postnatal development in agreement with previous reports (Lewis and Cowan, 1985; Tardy *et al.*, 1989) and morphologically defined astroglia types in the Juvenile and adult AGR.The limitation however is that GFAP immunohistochemistry can markedly underestimate the extent of astrocyte branching and territory in comparison with other means of detection such as Golgi staining or expression of reporter proteins such as GFP or β-galactosidase , or filling with fluorescent dyes.

Landry *et al.,* (1990) suggested the effect of age in the expression of astrocytes mRNA, a factor which will affect GFAP immunoreactivity intensity. This explains the the pattern observed in our study where neonates had limited expression of GFAP while the highest expressions were found in juvenile animals. Immunoreactivity was reduced in the adult group thus, indicating that aside the age of the animal determining the morphology and cellular population density observed in studies, it also determines the intensity of immunoreactions given equal values because of expressed levels of the protein GFAP across age groups. Literature also reported that age may also play a role in the degree of astrocytic coupling (Emsley and Macklis, 2006).

Astrocytic populations reveal that age influences the population of astrocytes in different regions of the brain. Higher astrocyte counts in juveniles than adults in most brain regions examined concurs with previous studies (Wang *et al.,* 2011) where certain regulatory proteins and mechanisms are known to cause astrocytic degeneration with increasing age

Significant difference in mean counts between juveniles and adult astrocytes were found in regions that can be described as association, commissural with little of white matter components (with the exception of the corpus callosum) indicating strong neuron-astrocyte interactions.

In this study, great amounts of GFAP immunoreactivity were seen in layers such as the periventricular layer (PL) of the olfactory bulb. It is known that the PL forms part of the rostral migratory stream (Ka´lma´n and Hajos 1989; Kosaka and Kosaka 2009) and as such may contain glia that aid in the tangential migration of newly generated cells from the SVZ to the olfactory bulb, and these may be the radial glia believed to facilitate radial migration of new cells to the glomerular layer where synaptic transmissions occur.

Morphological differences

In this study, 8-9 astrocytic subtypes were observed in the brain of the AGR. Several authors reported on variable subtypes, 9 in mice (Emsley and Macklis, 2010; Oberheim *et al.,*

2012), 4 in humans and primates (Nag, 2011). The cells were in distinct regional heterogeneity with respect to the type of astroglial cell that resides in each neuronally defined region of the adult CNS. It was found that distinct regions of the adult CNS, as defined 'classically' by neurons, can be characterized and defined by the presence of a few (in many cases as few as one) astroglial sub-types. The distinct, region-specific heterogeneity of astroglia is, thus, comparable to the regionalization and heterogeneity of defined neuronal subtypes.

There are distinct and region-specific differences in astroglial morphology in the AGR brain. These morphologic differences are as diverse as those for neuronal populations across the adult mammalian CNS, and can be used to define anatomical regions. Astroglial density varied considerably, and can distinctly and independently define neuronally-defined anatomical regions and sub-regions. Existing literature however suggest that the extent to which the varied morphology of astroglial sub-types and cellular density is seen depended on how astroglia are labeled and thus, other stains and techniques should be carried out in future analysis to differentiate or further elucidate the morphology and cell density. The study demonstrated that astroglial morphology and density independently define the discrete cytoarchitecture of the AGR brain especially in the juvenile.

OLIGODENDROCYTES

Various authors described classification properties of oligodendrocytes using different criteria. Rio Hortega (1928) classified oligodendrocytes in four categories, in relation to the number of their processes i.e. according to their morphology and the size or thickness of the myelin sheath they form, Butt *et al.,* (1995) also distinguished four types of myelinating oligodendrocytes, from small cells supporting the short, thin myelin sheaths of 15–30 small diameter axons (type I), through intermediate types (II and III), to the largest cells forming the long, thick myelin sheaths of one 1–3 large diameter axons. In this study, 5 stages of oligodendrocyte development were described based on dendritic development and myelinating status using Hortega's prototype to include precursor-progenitor, preoligodendrocytes, immature oligodendtrocytes, mature non-myelinating and matured myelinating oligodedntrocytes in the AGR. This is the first time Oligodendrocytes and their developmental stages were demonstrated in the AGR.

OLFACTORY BULB

The organization of the main olfactory bulb is largely typical as described for other mammals but there is no ependymal cell layer or olfactory ventricle as described in some other mammals e.g. sheep, rabbits and pigs Marschner (1970), Smitka *et al.*,(2009).The MOB was divided into 6 layers in this study but literature describes up to 8 layers (Marschner,1970) to include the ependymal layer and an external granule cell layer, located between the glomerular and external plexiform layers in certain species. Typically the ependymal and periventricular (or sub-ependymal) layers constitute one layer. However, in some mammals e.g. the African Elephant, the persistence of an olfactory ventricle means that a distinct population of ependymal cells is found lining the wall of the ventricle Ngwenya *et al.*, (2011).

The glomerular layer was a maximum of 2 layers density around the periphery of the bulb with obvious features of myelination. However, at the mid sagittal section, a central accentuation of glomeruli up to about 8 layers occurs. This feature is atypical in many mammals. Reports of up to 4 layers dense glomeular layers have been documented in the African Elephant Ngwenya *et al.*, (2011) but not the mid sagittal accentuation profile. The glomerular layer has a functional significance in that it serves as the site of synaptic contact between olfactory receptor axons and olfactory principal cells and acts as the functional units of the olfactory bulb (Chao *et al.*,1997). In most instances mitral cells show no positivity for myelin stains (Ngwenya *et al.*, 2011). Together with tufted cells, mitral cells are the major class of output cells of the bulb. They extend as a single apical dendrite through the EPL to the GL, where it arborizes extensively throughout much of a single glomerulus and are known to give off axon collateral which terminate, within the bulb, in the internal plexiform layer and GCL (Mori *et al.*,1983), Price and (Powell.1970), or exit the MOB and innervate a number of olfactory related brain regions collectively known as the piriform olfactory cortex (POC).There is evidence for extensions of newly generated neurons to the POC in the AGR (Olude *et al.*, 2014b) and thus, the mitral cells are proposed to be involved in this network.

The GCL has been described as the largest layer of the MOB (Ha´la´sz and Shepherd, 1983; Kratskin and Belluzzi. 2003and Kosaka and Kosaka, 2009). In the African giant rat, the GCL and PVL were the most extensive and contained extensive glia and neuronal reactivity like the African Elephant (Ngwenya *et al.*, 2011). The GCL is the deepest neuronal layer in the bulb, and it contains the largest number of cells. Most of the neurons of the GCL are the granule cells,

but there are also small numbers of Golgi cells, Cajal cells, and Blanes cells. These granule cells are inhibitory GABAergic cells that form dendrodendritic synapses with mitral and tufted cells in the EPL (Ennis *et al.*, 2007). It is also known that the neurons of the PVL appear to be important both to MOB development and to certain olfactory functions during adulthood (Ennis *et al.*, 2007). That these 2 layers were the largest in this study may contribute significantly to the olfactory prowess of the AGR.

Evidence exists that newly generated neurons migrate via the Rostral Migratory Stream (RMS) from the subventricular zone (SVZ) of the lateral ventricle to the olfactory bulb of rats and even the African giant rat to integrate into the glomerular and granule cell layers (Peretto *et al.*, 1997; Be´dard and Parent, 2004 and Lledo *et al.*, 2006). Doublecortin (DCX) and Ki-67stainings were used to visualize adult neurogenesis in the AGR MOB. The DCX fibres of the periventricular layers were observed to be tangentially orientated, and this is consistent with the proposal that newly generated cells initially migrate tangentially in the rostral migratory stream, going from the SVZ to the MOB (Lledo *et al.*, 2006).

Glia distribution in the AGR olfactory bulb did not differ from descriptions given for other mammals. GFAP immunoreactivity was observed in all layers of the MOB in the AGR which is typical with results obtained by (Chao *et al.*, 1997 and Ka´lma´n and Hajo´s, 1989) where immunoreactive glial cells were observed in all MOB layers of the rat, particularly the GCL and GL. In the African elephant, there was no GFAP in the EPL, and while GFAP-immunopositive glial processes were observed in the GCL, no glial cell bodies were immunopositive (Ngwenya *et al.*, 2011). In this study, great amounts of GFAP immunoreactivity were seen in layers such as the periventricular layer, granule cell layer and GL. It is known that the PVL forms part of the rostral migratory stream (Ka´lma´n and Hajo´s, 1989; Kosaka and Kosaka, 2009) and as such may contain glia that aid in the tangential migration of newly generated cells from the SVZ to the olfactory bulb and these may be the radial glia believed to facilitate radial migration of new cells to the glomerular layer where synaptic transmissions occur.

The expression of 2’ , 3’ -cyclic nucleotide 3 phosphodiesterase (CNPase), a protein related to axonal ensheathment by myelinating cells has been shown to follow a caudorostral pattern with the exception of the glomerular layer(Gomes *et al.*, 2003) with evidence that oligodendrocytes appear in the OB probably with some generated from the SVZ from post natal

day 5 and a *de novo* ensheathment with a report that there was no age-related loss of CNPase immunoreactivity in glial cell bodies as CNPase immunoreactivity in cell bodies has been shown to persist up to adult stage in the OB(Barradas *et al.*, 1995; 1998 and Gomes *et al.*, 2003). A possible explanation for the persistent expression in OB cell bodies is that it is linked to myelination of non-axonal segments of newly generated neurons (periglomerular or granular cells) and or of cells that change their synaptic partners (mitral and tufted cells) often, as suggested by (Remahl and Hildebrand, 1985). Thus in this study, CNPase confirms myelination in different regions of the MOB with its resultant effect of potentiation at the glomerular synapses in the AGR. It is also remarked in literature that non-axonal myelination has similar features in normal OB and in some partially denervated structures, such as the lateral geniculate nucleus of decorticated animals, in which there was elimination and *de novo* formation of synapses (Gomes *et al.*, 2003). It is possible that plastic phenomena, such as *de novo* formation of synapses, create and or maintain a microenvironment that favors the generation of new oligodendrocytes and or their maintenance in a relatively immature condition, with persistent *de novo* ensheathment of neurons (Gomes *et al.*, 2003).

Olfactory function in AGR

A number of features indicated the importance of olfaction in AGR including the relatively large olfactory bulb and its present day use in landmine detection and tuberculosis diagnosis (Verhagen *et al.*, 2003; Weetjens *et al.*, 2009). Though the APOPO group has utilized this rodent by training and utilizing the olfactory powers, there has been little description of the neurocellular or chemical make-up of the olfactory system in AGR (Nzalak *et al.*, 2005; Igbokwe and Nwogu, 2009). There was the presence of an accessory olfactory bulb in the AGR which may likely be responsible for chemical signalling. The vomeronasal organ is responsible for the reception of non-volatile pheromonal chemical stimuli and the transduction of these signals to the AOB via the vomeronasal nerve (Halpern, 1987). Though chemical communication in AGR has not been well documented it is believed to be vital to its social behaviour e.g. to strengthen female bonds and maternal bonds between dam and offspring. More so, the female AGR is observed to be poor in foster mothering as it eats up the neonate from other dams (Cooper, 2008). To further strengthen this, Ki-67 and DCX immunopositive cells have been observed in the somatosensory cortex of the brain of the juvenile AGR. This may be due to progenitors

derived from the subventricular germinal zone and or local parenchyma progenitor and this feature, is proposed to be associated with the social lifestyle with its continuous burrowing which indicate a motor function of the brain and memory. This pattern has been proposed in other rodents with similar neurogenic profile in potential sites (Luzzati *et al.*, 2007). This may explain the use of AGR in landmine detection and tuberculosis diagnosis. However, further work is required to confirm the chemical neuroanatomy and physiology of the AGR OB to understand the principles of olfaction in this species and maximize its use.

This study served as a pilot study into the cellular architecture of the olfactory bulb of the AGR and further research is needed to determine the chemical and physiological neuroanatomy in a bid to better understand how the animal uses its olfactory powers to sniff out landmines and diagnose tuberculosis a quality which may be put to other useful purposesi.

ADULT NEUROGENESIS

Reliability of DCX and Ki-67 as markers of adult neurogenesis

One of the approaches to identifying dividing cells in the brain of mammals involves immunostaining of specific proteins that are expressed only during cell divisions (e.g. Ki-67, which is only present during the active phases of the cell cycle) (Amrein *et al.*, 2004) or proteins that are only present in recently generated and still-developing cells of various types e.g. DCX, a microtubule associated protein expressed in the cytoplasm of recently generated cells that differentiate into neurons (Matsuo *et al.*, 1998). In these still-developing young neurons, DCX is present in the cytoplasm, including axons (Francis *et al.*, 1999; Gleeson *et al.*, 1999). In the brain of adult mammals, DCX is transiently expressed in recently generated cells that migrate and differentiate into neurons (Cai *et al.*, 2009; Liu *et al.*, 2008; Xiong *et al.*, 2008; Luzzati *et al.*, 2009). Reliability of DCX as a marker of neuroblasts and immature neurons has been validated in the adult mammalian brain with double labeling for BrdU and DCX indicating that DCX is only transiently expressed in developing neurons of the adult brain (Kempermann *et al.*, 2003; Grabiec *et al.*, 2009; Brown *et al.*, 2003). However, it has been hypothesized that DCX expression could recur in mature neurons if neuronal plasticity leading to reorganization of microtubules is induced, for example during synaptic reorganization or axonal outgrowth (Nacher *et al.*, 2001). This hypothesis implies that the piriform cortex is a very plastic area, and

therefore the continued expression or re-expression of DCX in its neurons could be one of the mechanisms of plastic changes.

DCX and Ki-67 positive cells in the potential neurogenic sites

The presence of cells expressing DCX in the somatosensory and piriform cortices, especially in layer II, has been first observed in the rat (Nacher *et al.*, 2001; Brown *et al.*, 2003; Shapiro *et al.*, 2007; Klempin *et al.*, 2011). Several groups confirmed these observations independently in mice (Cameron and Dayer 2008; Kokoeva *et al.*, 2007), and cats and primates (Cai *et al.*, 2009; Alvarez-Buylla and Garcia-Verdugo 2002; Luzzati *et al.*, 2007). With our results, AGR can be added to the list of rodents which also demonstrated DCX immunopositivity. In some juvenile rats investigated, the anterior commissure contained DCX-labelled processes. These could belong to DCX-expressing neurons of either OB or the piriform cortex, or both, as both (Fox *et al.*, 1948; Patzke *et al.*, 2013), reported that newly generated neurons are able to send long-range projections into the adult brain. The piriform cortex receives direct input from the OB (Scott *et al.*, 1980) and in all examined species of mammals; the OB shows the highest rate of incorporation and turnover of the new neurons throughout life. Indicating a constant exchange of neurons takes place, allowing for an extraordinary plasticity of the whole olfactory system. Most of the positive DCX cells are believed to migrate to the olfactory bulb and incorporate there (Bartkowska *et al.*, 2010). Some may deviate from this track towards the frontal cortex between layers II and III (Biebl *et al.*, 2000). A review by Ihunwo and Pillay (2007) reported the potential sites to include the septum and striatum, substantianigra, third ventricle, spinal cord, amygdale, cerebral cortex, dorsal vagal complex but not the juvenile cerebellum as observed in this study.

Neurogenesis across the age groups of African giant rat

In this study, we showed for the first time the presence of newly generated cells in brains of neonate, juvenile and adult African giant rats, a species belonging to the sub order-Myomorpha , order rodentia. DCX-labelled neurons were present in the DG, SVZ of the lateral ventricles to include the RMS, OB, anterior commissure, striatum somatosensory and piriform cortices, and their labelled axons were extending to the anterior commissure in some juvenile rats, while proliferating cells expressing Ki-67 were observed only in limited regions of the

brain: the SVZ of the lateral ventricles (including the olfactory bulb), RMS, DG, the cerebellum and cortex in juvenile AGR.

Age group classification was judged by already reported parameters which include their weight and sexual maturity (Ajayi 1974). This study was more qualitative but exact neuronal quantities were determined in the SVZ and DG by counting the proliferative cells staining ki-67 positive. Cells clearly showed that the rate of neurogenesis was higher in juveniles and lowest in adults . This pattern has been reported, that adult neurogenesis depends on strain and age of rats and mice (Kempermann *et al.,* 1997) with the AGR showing increase in adult neurogenesis from neonates to adults.

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 (Eriksson *et al.,* 1998; Grabiec *et al.,* 2009; Maslov *et al.,* 2004; Kuhn *et al.,* 1996). However, it has been reported in rats that the number of cells generated in the DG of the Hippocampus decreased significantly before their midlife but afterwards the rate of neurogenesis in their DG remained at the same level throughout the rest of their life (Kuhn *et al.,* 1996). The AGR did not reveal this pattern but had an increase in cell counts toward juvenile life and sharply decreased towards adult life. Among mice, the highest rate of adult neurogenesis was found in wood mice as compared with the bank voles and pine voles (Amrein *et al.,* 2004), while in rodents from the Sciuridae family, the rate of adult neurogenesis was lower in chipmunks than in squirrels (Barker *et al.,* 2005). In some species of bats and Sorex shrews, adult hippocampal neurogenesis was completely absent in the DG (Bartkowska *et al.,* 2008; Amrein *et al.,* 2007). However, Chawana *et al.,* (2013) recently reported AHN in all 8 megabats investigated.

The role of adult hippocampal neurogenesis in some behavioural patterns of the African giant rat

The role of adult hippocampal neurogenesis in spatial memory is highly debated (Amrein and Lipp 2009). DCX staining revealed a dense status of neurogenesis especially in the juvenile. 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 DCX staining at all levels of the olfactory cells. Also, the presence of positive cells in the piriform and somatosensory cortices 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).The immunopositive cells observed in the somatosensory cortex of the brain of the juvenile AGR may be due to progenitors derived from the subventricular germinal zone and or local parenchyma progenitor. However, the presence of immature neurons in the somatosensory and piriform cortices of AGR may be associated with the social lifestyle and its complex burrows which indicate brain functions that may be associated with memory. The continuous burrowing also indicates high motor functions and has been proposed in other rodents with similar neurogenic profile in potential sites (Luzzati *et al.*, 2007). It is left to be determined if the somatosensory specialization of the AGR obscures the importance of the olfactory system. Nonetheless, it is believed that the AGR obtains its prey using olfactory cues rather than gustation. In conclusion, this study reveals a high level of plasticity in the AGR and juveniles in particular, in which we hypothesize that juveniles have the best learning abilities in this species.

CONCLUSION

This study investigated the neurocellular profile and adult neurogenesis of the African giant rat (*Cricetomys gambianus*, Waterhouse) by utilizing H&E and Nissl for cytoarchitectural orientation, Kluver and Barrera and Silver impregnation methods as differential stains for myelin sheath and neuron localization respectively. Free floating Immunohistochemical technique was carried out using antibodies against glial fibrillary acidic protein (GFAP) for astrocyte localization, 2' 3' cyclic nucleotide phosphodiesterase (CNPase) for oligodendrocyte and myelin localization, Ionizing calcium binding adapter molecule (IbA1) for microglia localization, Doublecortin (DCX) for immature and migration neurons and Ki-67 for proliferating neurons. Findings from this study have shown to contribute to knowledge in several ways including the discovery of the falx cerebri insertion in between the main olfactory bulb in the AGR, description of the cortical neuron types, glia morphology and heterogeneity, multilayered ependemocytes in the AGR (adult and juvenile) ventricular lining, cytoarchitecture of the main olfactory bulb, mullti-layering in the mid central glomerular layer of the olfactory bulb and the existence and sites of adult neurogenesis in the AGR. It bears record that none of these findings have been recorded in literature before now. They have application in understanding the structural and functional capacities of the brain of this indigenous rodent. Further work will be required to study the chemical neuroanatomy of various components of this rodent.

REFERENCES

Adams, N. C., Tomoda, T., Cooper, M., Dietz, G. and Hatten, M. E.. (2002). Mice that lack astrotactin have slowed neuronal migration. *Development* 129: 965–972.

Adeyemo, O. and Oke, B.O. (1990). Comparison of the testicular and epididymal proteins of the African giant rat (*Cricetomys gambianus*, Waterhouse) and the Laboratory rat. *Tropical Veterinarian*, 8: 17-27.

Agduhr, E. (1932). Cytology and cellular of nervous system. In: Choroid plexus and ependyma. W.Penfield (ed). P.B. Hoeber Inc. New York, Vol. cii pp: 536 - 573.

Ajayi, S.S. (1974). The biology and domestication of African giant rat (*Cricetomys gambianus* Waterhouse), Ph.D. Thesis.

Ajayi, S., (1977a). Field observations on the African giant rat *Cricetomys gambianus* in southern Nigeria. *East African Wildlife Journal*, 15(3): 191-198.

Ajayi, S. (1977b). Live and carcass weight of giant rat (*Cricetomys gambianus*) and domestic rabbit (*Ortyctalagus cuniculus*). *East African wildlife journal, 15*(3): 223-228.

Ajayi, S., Tewe, O. and Faturoti, E. (1978). Behavioral changes in African giant rat (*Cricetomys gambianus*, Waterhouse) under domestication. *East African Wildlife Journal*, 16(2): 137-143.

Ajayi, S.S. (1975). Caging and breeding the African giant rat (*Cricetomys gambianus*, Waterhouse). *Journal of the Institute of Animal Technicians*, 25(2): 75-81.

Akinloye, A.K. (2009). Structural and hormonal studies of the female giant rats (*Cricetomys gambianus*, Waterhouse) at different stages of oestrous cycle: PhD thesis

Akinloye, A.K. and Oke, B.O. (2009b). Gross Morphometry of Ovary of the Female African Giant Rat (*Cricetomys gambianus*, Waterhouse) At Different Stages of the Oestrous cycle. *Tropical Veterinarian*, 27(1): 10 - 16.

Alder, J., Lee, K. J. Jessell. T. M.and Hatten. M. E.(1999). Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells. *Nature Neurosci.* 2: 535–540.

Ali M. N., Byanet O., Salami S. O., Imam J., Maidawa S. M., Umosen A. D., Alphonsus C. and Nzalak J. O. (2008). Gross anatomical aspects of the gastrointestinal tract of the wild African giant pouched rat (*Cricetomys gambianus*). *Scientific Research and Essay.* 3 (10): 518-520.

Ali M.N., Onyeanusi, B.I. Ojo, S.A. Ayo, J.O. Maidawa S.M.and Imam, J. (2010). Biometric and morphologic studies of the female reproductive organs of the African giant rat (*Cricetomys gambianus*, Waterhouse). *Folia Morphol.* 69(4): 213–215.

Alonso Mariana, Ce´cile Viollet, Marie-Madeleine Gabellec,Vannary Meas-Yedid,Jean-Christophe Olivo-Marin and Pierre-Marie Lledo (2006) Olfactory Discrimination Learning Increases the Survival of Adult-Born Neurons in the Olfactory Bulb 1. *The Journal of Neuroscience*, 26(41):10508 –10513

Alvarez-Buylla A, Garcia-Verdugo JM (2002). Neurogenesis in adult subventricular zone. *J Neurosci*, 22:629–634.

Alvarez-Buylla, A., and Lim, D.A. (2004). For the long run: maintaining germinal niches in the adult brain. Neuron 41, 683–686.

Amenta F, Del Valle M, Vega J A and Zaccheo D (1991). Age-related structural changes in the rat cerebellar cortex: Effect of choline alfoscerate treatment; *Mech. Ageing Dev.* 61 173–186

Amrein I, Dechmann DK, Winter Y, Lipp HP (2007). Absent or low rate of adult neurogenesis in the hippocampus of bats (Chiroptera). *PLoSONE* 2:e455

Amrein I, Lipp HP (2009). Adult hippocampal neurogenesis of mammals:evolution and life history. *Biol Lett* 5:141–144

Amrein, I., Slomianka, L., Poletaeva, I.I., Bologova, N.V. and Hans-Peter, L. (2004). Marked species and age-dependent differences in cell proliferation and neurogenesis in the hippocampus of wild-living rodents. *J.Hipp.* 14:1000-1010.

Andersen B B, Gundersen H J and Pakkenberg B (2003). Aging of the human cerebellum: A stereological study; *J. Comp. Neurol.*466:356–365

Araque A, Carmignoto G and Haydon PG (2001). Dynamic signaling between astrocyte and neurons; *Annu. Rev. Physiol.* 63: 795–813

Asibey, E.O.A. and Addo, P.G. (2000). The Grasscutter; a promising animal for meat production. In: Turnham D, (editor) African perspectives. Practices and policies supporting sustainable development.(Scandinavian Seminar College, Denmark, in association with Weaver press, Harare, Zimbabwe).pp 120.

Asojo T.A and Aire, T.A. (1983). Microstereological and histochemical studies of the salivary glands of the giant rat (*Cricetomys gambianus*, Waterhouse).*ActaAnat (Basel)*, 117:65-72.

Attar A, Kaptanoglu E, Aydin Z, Ayten M, Sargon MF. (2005). Electron microscopic study of the progeny of ependymal stem cells in the normal and injured spinal cord. *Surg Neurol;64 (Suppl 2)*:28-32.

Attwell P. J., Cooke S. F. and Yeo, C. H. (2002). Cerebellar function in consolidation of motor memory; *Neuron* 34:1011–1020

Bakalian, A., Corman, B., Delhaye-Bouchaud, N. and Mariani, J. (1991).Quantitative analysis of the Purkinje cell population during extreme ageing in the cerebellum of the Wistar/Louvain rat; *Neurobiol. Aging* 12:425–430

Bannerman, D.M., Rawlins, J.N., McHugh, S.B., Deacon, R.M., Yee, B.K.,Bast, T., Zhang, W.N., Pothuizen, H.H., and Feldon, J. (2004). Regional dissociations within the hippocampus–memory and anxiety.*Neurosci.Biobehav.Rev.* 28, 273–283.

Barker JM,Wojtowicz JM, Boonstra R (2005). Where's my dinner? Adult neurogenesis in free-living food-storing rodents. *Genes Brain Behav* 4:89–98

Barradas PC, Gomes SS, Cavalcante LA (1995). CNPase expression in the developing opossum brain stem and cerebellum. *NeuroReport* 6:289–292

Barradas PC, Gomes SS, Cavalcante LA (1998). Heterogeneous patterns of oligodendroglial differentiation in the forebrain of the opossum Didelphis marsupialis. *J Neuro Cytol* 27:15–25

Bart Rypma, Vivek Prabhakaran, John E. Desmond, and John D. E. Gabriel, (2001). Age Differences in Prefrontal Cortical Activity in Working Memory. *Psychology and Aging.* 16, (3): 371-384

Bartkowska K, Djavadian RL, Taylor JR, Turlejski K (2008). Generation, recruitment and death of brain cells throughout the life cycle of Sorex shrews (Lipotyphla). *Eur J Neurosci* 27:1710–1721

Bartkowska, K., Turlejski, K., Grabiec, M., Ghazaryan, A., Yavruoyan, E and Djavadian, R.L. (2010). Adult neurogenesis in the Hedgehog (Erinaceus concolor) and Mole (Talpa euroapaea). *Brain Behav Evol*, 76:128-143.

Bastianelli, E., K. Moutairouand M.T. Akele-Akpo, (1999). Calcium Binding Proteins Immunohistochemistry and Identification of Neurons in the Mammalian Pineal Gland of the African Giant Rat: *Cricetomys gambianus, Gen Physiol Biophys* 18, 5-17.

Be´dard A. and Parent A. (2004). Evidence of newly generated neurons in the human olfactory bulb. *Brain Res Dev Brain Res* 151:159–168

Bell, S.D (2004). "Aplodontid, Scuirid, Castorid, Zapodid and Geomyoid rodents of the Rodent Hill locality, Cypress Hills Formation, Southwest Saskatchewan" (M.Sc. thesis, Geological Sciences, University of Saskatchewan, Saskatoon).

Bianchi Serena, Amy L. Bauernfeind, Kanika Gupta, Cheryl D. Stimpson,Muhammad A. Spocter, Christopher J. Bonar, Paul R. Manger, Patrick R. Hof, Bob Jacobs, and Chet C., Sherwood (2011). Neocortical neuron morphology in Afrotheria: comparing the rock hyrax with the African Elephant. *Ann. N.Y. Acad. Sci.* 1225 (2011) 37–46

Biebl, M., Cooper, C.M., Winkler, J. and Kuhn, G.H. (2000). Analysis of neurogenesis and programmed cell death reveals a self renewing capacity in the adult rat brain. *Neursci.lett.* 291: 17-20.

Blakemore Sarah-Jayne and Choudhury Suparna (2006). Development of the adolescent brain: implications for executive function and social cognition, *Journal of Child Psychology and Psychiatry* 47 (3):296–312

Bobe, L., Mabela. M. (1997). Incidence of four gastro-intestinal parasite worms in group of cricetomas, *Cricetomys gambianus* (Rodent: Cricetidae), caught it Lukaya- Democratic Republic of Congo. *Tropicultura*, 15(3): 132-135.

Böhme, G., and Franz, B. (1967). Die binnenraume des gehirns von ratte und maus. *ActaAnat*.68: 199-206. 1967

Boyden ES, Katoh A and Raymond JL, (2004). Cerebellum-dependentlearning: The role of multiple plasticity mechanisms; *Annu.Rev. Neurosci.* 27:581–609

Brauer, K., and W. Schober. (1970). Qualitative und quantitative untersuchungen am corpus geniculatum laterale (Cgl) der laborratte: I. Zur struktur des cgl unter besonderer Berucksichtigung der golgi-Architecktonik. *J. Hirnforsch*. 14: 389-398. 1973.

Breunig, J.J., Macklis, J.D., and Pakic, P. (2007). Evolving methods for the labelingand mutation of postnatal neuronal precursor cells: a critical review. In Adult neurogenesis, F.H. Gage, G. Kempermann, and S. Hongjun, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 49–80.

Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H,Winkler J,.(2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur. J. Neurosci.* 17:2042–46

Bruel-Jungerman, E., Laroche, S., and Rampon, C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur. J. Neurosci*. 21, 513–521.

Bruijn LI, Miller TM, Cleveland DW (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annual Reviews of Neuroscience*, 27:723–749.

Bruni JE, Del Bigio MR, Clattenburg RE. (1985). Ependyma: normal and pathological. A review of the literature. *Brain Res.*356(1):1-19.

Bryant SV. and Wozney KJ. (1974). Stimulation of limb regeneration in the lizard Xantusia vigilis by means of ependymal implants. *J Exp Zool.*189:339-352

Bushong EA, Martone ME, Jones YZ, Ellisman MH.(2002). Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *Journal of Neuroscience.* 22:183–192

Butt AM, Ibrahim M, Ruge FM, and Berry M. (1995). Biochemical subtypes of oligodendrocyte in the anterior medullary velum of the rat as revealed by the monoclonal antibody Rip. *Glia* 14: 185–197.

Byanet, O., Onyeanusi, B. I. and Ibrahim, N. D. G. (2009). Sexual dimorphism with respect to the macro-morphometric investigations of the forebrain and cerebellum of the Grasscutter (*Thryonomys swinderianus*). *Int. J. Morphol., 27(2)*:361-365.

Cai Y, Xiong K, Chu Y, LuoDW, LuoXG,Yuan XY, Struble RG, CloughRW, Spencer DD, Williamson A, Kordower JH, Patrylo PR, YanXX (2009). Doublecortin expression in adult cat and primate cerebral cortex relates to immature neurons that develop into GABAergic subgroups. *Exp Neurol* 216:342–356

Callebaut, M. and Van Nassauw, L. (1987). Immunohistochemical demonstration of actin and desmin by monoclonal antibodies in the ovary of the rat. *Med. Sci. Res*. 15; 557–558.

Camain, R. and Quenum, A. (1961).The problem of compensatory hypertrophy of the adrenal cortex: study in the male *Cricetomys gambianus*. *C R Seances Soc Biol Fil*.; 155:585-591.

Cameron HA, Dayer AG (2008). New interneurons in the adult neocortex: small, sparse, but significant? *Biol Psychiatry* 63:650–655

Cao, L., Jiao, X., Zuzga, D.S., Liu, Y., Fong, D.M., Young, D., and During, M.J.(2004). VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat. Genet*. 36, 827–835.

Capela, A., and Temple, S. (2002). LeX/ssea-1 is expressed by adult mouseCNS stem cells, identifying them as nonependymal. *Neuron* 35, 865–875.

Cartmill, Matt (1987)..Human Structure. Cambridge: Harvard University Press,

Cassina P, Pehar M, Vargas MR, Castellanos R, Barbeito AG, Estevez AG, Thompson JA, Beckman JS, Barbeito L. (2005). Astrocyte activation by fibroblast growth factor-1 and motor neuron apoptosis: implications for amyotrophic lateral sclerosis. *Journal of Neurochemistry.* 93:38–46.

Caston J, Hilber P, Chianale C and Mariani J (2003). Effect of training on motor abilities of heterozygous staggerer mutant (Rora (+)/Rora (sg)) mice during aging; *Behav. Brain Res.* 141: 35–42

Chaline J, Mein P, Petter F. (1977). Les grandes lignes d'une classification évolutive des Muroidea. *Mammalia* 41: 245– 252.

Chao TI, Kasa P, Wolff JR (1997). Distribution of astroglia in theglomeruli of the rat main olfactory bulb: exclusion from thesenso.training operations and odour detection. Geneva: Geneva International Centre for Humanitarian Demining, pp 175–193.

Chapouton, P., Jagasia, R., and Bally-Cuif, L. (2007). Adult neurogenesis in non-mammalian vertebrates. *Bioessays* 29, 745–757.

Chawana R, Patzke N, Kaswera C, Gilissen E, Ihunwo AO, Manger PR (2013). Adult neurogenesis in eight Megachiropteran species. *Neuroscience* 244:159–172

Codjia JTC, Chrysostome C, Civitelli MV, Capanna E.(1994). Les chromosomes des rongeurs du Bénin (Afrique de l'ouest). I: Cricetidae. *Rendiconti Lincei* 9: 277–287.

Cohrs, P. (1936). Das subfornikale organs des 3.Ventrikels. Nach untersuchungen bei den Haussaugetieren, einigen nagetieren und dem menschen. *Z. Anat. Entwicklungsgesch*. 105: 491-518. 1936.

Consiglio A, Gritti A, Dolcetta D, Follenzi A, Bordignon C, Gage F, Vescovi AL, Naldini L. (2004). Robust in vivo gene transfer into adult mammalian neural stem cells by lentiviral vectors. *Proc Natl Acad Sci USA.* 101:14835–14840.

Cooper RG. (2008). Care, husbandry and diseases of the African giant rat (*Cricetomys gambianus*). *Journal of the South African Veterinary Association* 79(2): 62–66

Corti M, Castiglia R, Colangelo P, Capanna E, BeolchiniF, Bekele A, Oguge NO, Makundi RH, Sichilima AM,Leirs H, Verheyen W, Verhagen R. (2005). Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia.*Belgian Journal of Zoology* 135 (Suppl): 197–216.

D'Ambrosio R, Wenzel J, Schwartzkroin PA, McKhann GM, Janigro D. (1998). Functional specialization and topographic segregation of hippocampal astrocytes. *Journal of Neuroscience.*18:4425–4438.

Davis S, Thomas A, Perry R, Oakley A, Kalaria R N and O'Brien J T (2002). Glial fibrillary acidic protein in late life major depressive disorder: An immunocytochemical study; *Exp. Toxicol.Pathol.*54 31–38

Deisseroth, K., Singla, S., Toda, H., Monje, M., Palmer, T.D., and Malenka, R.C. (2004). Excitation-neurogenesis coupling in adult neural stem progenitor cells. *Neuron* 42, 535–552.

Dempsey M.A (1991). Cranial Foramina and Relationships of Dipodoid Rodents. Submitted to the Committee on Undergraduate Honors of Baruch College of The City University of New York in partial fulfillment of the requirements for the degree of Bachelor of Arts in Biology with Honors

Dipeolu, O. and S. Ajayi, (1976). Parasites of the African giant rat *Cricetomys gambianus* in Ibadan Nigeria. *East African Wildlife Journal*, 14(1): 85-89.

Dipeolu OO, Akinbode OA, Ogunji F (1981). Observation on the African giant rat *(Cricetomys gambianus).Bull. Anim. Health Prod. Afr.*, 29: 393-397.

Dobigny G, Nomao A, Gautun JC. (2002). A cytotaxonomic survey of Rodents from Niger: implications for systematics, biodiversity and biogeography. *Mammalia* 66: 495–523.

Dobrossy, M.D., Drapeau, E., Aurousseau, C., Le Moal, M., Piazza, P.V., and Abrous, D.N. (2003). Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. *Mol. Psychiatry* 8, 974–982.

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97:703–16

Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A. (2002). EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* 36:1021–34

Drapeau, E., Montaron, M.F., Aguerre, S., and Abrous, D.N. (2007). Learning induced survival of new neurons depends on the cognitive status of aged rats. *J. Neurosci.* 27, 6037–6044.

Duan, X., Chang, J.H., Ge, S., Faulkner, R.L., Kim, J.Y., Kitabatake, Y., Liu, X.B., Yang, C.H., Jordan, J.D., Ma, D.K., (2007). Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. *Cell* 130, 1146–1158.

Dubois J-Y, Rakotondravony D, Hanni C, Sourouille P, Catzeflis FM. (1996). Molecular evolutionary relationships of three genera of Nesomyinae, endemic rodent taxa from Madagascar. *Journal of Mammalian Evolution* 3: 239–259.

Dupret, D., Fabre, A., Dobrossy, M.D., Panatier, A., Rodriguez, J.J., Lamarque, S., Lemaire, V., Oliet, S.H., Piazza, P.V., and Abrous, D.N. (2007). Spatial learning depends on both the addition and removal of new hippocampal neurons. *PLoS Biol.* 5:e214.

Duru FIO, Ajayi S, Azu OO (2013). The effect of unilateral vasectomy on testosterone and testicular parameters in the adult male African giant rat (Cricetomys gambianus) *African Health Sciences* 2013; 13(2): 483 - 489

Dwarika Sarika, Busisiwe C. Maseko, Amadi O. Ihunwo,Kjell Fuxe, Paul R. Manger, (2008). Distribution and morphology of putative catecholaminergic and serotonergic neurons in the brain of the greater canerat, Thryonomys swinderianus, *Journal of Chemical Neuroanatomy* 35: 108–122

Edmondson, J. C., R. K. H. Liem, J. E. Kuster and M. E. Hatten. (1988). Astrotactin: A novel neuronal cell surface antigen that mediates neuronal-astroglial interactions in cerebellar microcultures. *J. Cell Biol.* 106: 505–517.

El-Bassouny, D. R. and Hindawy, M. F.,(2006). Strutural relationship of the ependymocytes and their processes within the thoracic spinal segments in albino rat, *the Egyptian journal of histology vol.* 29. *No.2,* 327 - 338.

Ellerman, J.R. (1941):. *The families and genera of living rodents. Family Muridae*. London Printed by order of the Trustees of the British Museum. *Vol. 2.*

Emsley JG. and Macklis JD. (2006). Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. *Neuron Glia Biol;* 2 (3):175-186.

Ennis M, Hamilton KA, Hayar A (2007). Neurochemistry of the mainolfactory system. In: Lajtha A (ed) Handbook of neurochemistryand molecular neurobiology, 3rd edn. Springer, New York pp139-182

Eric, H. C. (2006). *Brain facts and figures. In*: Neurosciences for Kids. Washington, University of Washington, pp.1-8.

Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C and Peterson, D.A. (1998).Neurogenesis in the adult human hippocampus. *Nat. Med.* 4:1313-1317.

Esposito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., and Schinder, A.F. (2005). Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J. Neurosci.* 25, 10074–10086.

Feldhamer, G.A. C.D. Lee, S.H. Vessey and J.F. Merritt (1999). *Mammalogy*.New York, WCB, McGraw-Hill. pp 563

Fishell, G. and M. E. Hatten. (1991). Astrotactin provides a receptor system for CNS neuronal migration. *Development* 113: 755–765.

Fjellanger, R., (2003)."The REST (Remote Explosive Scent Tracing) Concept." In GICHD (ed.), Mine Detection Dogs: Training, Operations and Odour Detection, GICHD,Geneva.

Fox CA, Fischer RR, Desalva SJ (1948). The distribution of the anterior commissure in the monkey, Macacamulatta. *J Comp Neurol* 89:245–277

Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC,Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK,Berwald-Netter Y, Denoulet P, Chelly J (1999). Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron* 23:247–256

Frantz, G. D., J. M. Weimann, M. E. Levin and S. K. McConnell. (1994). Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. *J. Neurosci*. 14: 5725–5740.

Frantz, G. D. and McConnell S. K.. (1996). Restriction of late cerebral cortical progenitors to an upper-layer fate. *Neuron* 17: 55–61.

Fukuchi-Shimogori, T. and E. A. Grove. (2001). Neocortex patterning by the secreted signaling molecule FGF8. *Science* 294: 1071–1074.

Fukuchi-Shimogori, T. and E. A. Grove. (2005). Fibroblast growth factor 8 regulates neocortical guidance of area-specific thalamic innervation. *J. Neurosci.* 25: 6550–6560.

Fukuda, S., Kato, F., Tozuka, Y., Yamaguchi, M., Miyamoto, Y., and Hisatsune, T. (2003).Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. *J. Neurosci.* 23, 9357–9366.

Gage F. H (2000). Mammalian neural stem cells. *Science* 287:1433–1438

Gaiano, N. (2008). Strange bed fellows: Reelin and Notch signaling interact to regulate cell migration in the developing neocortex. *Neuron* 60: 189–191.

Garcia, A.D., Doan, N.B., Imura, T., Bush, T.G., and Sofroniew, M.V. (2004). GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat. Neurosci.* 7, 1233–1241.

Ge, S., Goh, E.L., Sailor, K.A., Kitabatake, Y., Ming, G.L., and Song, H. (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 439, 589–593.

Ge, S., Yang, C.H., Hsu, K.S., Ming, G.L., and Song, H. (2007). A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* 54, 559–566.

Geinisman Y, Bondareff W and Dodge JT (1978). Hypertrophy of astroglial processes in the dentate gyrus of the senescent rat; *Am. J. Anat.* 153 537–543

Genest-Villard H. (1967). Revision du genre *Cricetomys*(Rongeurs, Cricetidae).*Mammalia* 31: 390–455.

German DC, Liang CL, Song T, Yazdani U, Xie C and DietschyJM, (2002). Neurodegeneration in the Niemann–Pick C mouse:Glial involvement; *Neuroscience* 109 437–450

Ghashghaei, H.T., Weimer, J.M., Schmid, R.S., Yokota, Y., McCarthy, K.D., Popko, B., and Anton, E.S. (2007). Reinduction of ErbB2 in astrocytes promotes radial glial progenitor identity in adult cerebral cortex. *Genes Dev.* 21, 3258–3271.

Gilbert Scott F. (2010). Developmental biology. Ninth edition, copyright© 2010 by sinauer Associate, inc. ISBN 978-0-87893-384-6.

Gleeson JG, Lin PT, Flanagan LA, Walsh CA (1999). Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron* 23:257–271.

Gomes SS, Sergio LC, Marcelo FS, Luciane BL, Penha CB, Leny AC (2003). Expression of 2',3'cyclic nucleotide 3-phosphodiesterase(CNPase) in the developing olfactory bulb and subventricular zone rostral extension. *J Neurosci Res* 73:471–480

Gong, C., Wang, T.W., Huang, H.S., and Parent, J.M. (2007). Reelin regulates neuronal progenitor migration in intact and epileptic hippocampus. *J. Neurosci.* 27, 1803–1811.

Götz, M. and Y. A. Barde. (2005). Radial glial cells defined and major intermediates between embryonic stem cells and CNS neurons. *Neuron* 46: 369–372.

Gould, E. (2007). How widespread is adult neurogenesis in mammals? *Nat. Rev. Neurosci*. 8, 481–488.

Grabiec M, Turlejski K, Djavadian RL (2009). The partial 5-HT1A receptor agonist buspirone enhances neurogenesis in the opossum (Monodelphis domestica). *Eur Neuro psych pharmacol* 19:431–439

Granjon L, Duplantier JM, Catalan J, Britton-Davidian J. (1992). Karyotypic data on rodents from Senegal. *IsraelJournal of Zoology* 38: 263–276.

Greene, E. (1959).Ch. Anatomy of the rats.Ed. 2. New York: Hafner publishing co.

Gregg, J. M., and A. D. Dixon. (1973). Somatotropic organization of the trigeminal ganglion in the rat. *Arch. Oral. Biol*. 18: 487-498. 1973.

Guth, L. (1957). The effect of glossopharyngeal nerve transection on the circumvallate papillae of the rat. *Anat. Rec*. 128: 715-731. 1957.

Guthrie, S. and A. Lumsden. (1991). Formation and regeneration of rhombomere boundaries in the developing chick hindbrain. *Development* 112: 221–229.

Ha´la´sz N, and Shepherd GM (1983). Neurochemistry of the vertebrate olfactory bulb. *Neuroscience* 10:579–619

Hadj-Sahraoui N, Frederic F, Zanjani H, Delhaye-Bouchaud N,Herrup K and Mariani J, (2001). Progressive atrophy of cerebellar PC dendrites during aging of the heterozygous staggerer mouse (Rora+/sg); *Brain Res. Dev. Brain Res.* 126 201–209

Halpern M (1987). The organization and function of the vomeronasal system. *Annu Rev Neurosci* 10:325–362.

Hanashina, C., S. C. Li, L. Shen, E. Lai and G. Fishell. (2004). Foxg1 suppresses early cortical cell fate. *Science* 303: 56-59

Happold, D.C.D., (1987) The Mammals of Nigeria. Clarendon Press. Oxford.

Hara H, Onodera H, Kato H and Kogure K (1992). Effects of aging on signal transmission and transduction systems in the gerbil brain: Morphological and autoradiographic study; *Neuroscience*46 475–488

Harrington, M. J., E. Hong and R. Brewster. (2009). Comparative analysis of neurulation: First impressions do not count. *Mol. Reprod. Dev*. 76: 954–965.

Hatten, M. E. (1990). Riding the glial monorail: A common mechanism for glial-guided neuronal migration in different regions of the mammalian brain.*Trends Neurosci.* 13: 179-184.

Hebel R, Stronberg M (1975). Anatomy of the laboratory rat. Wilkins and Wilkins, 428 E. Preston Street, Baltimore, MD, USA.Library of Congress cataloging in publication data.

Hebel R. and Stronberg M. (1976). Anatomy of the laboratory rat; The Wilkins and Wilkins company, 428 E. Preston Street, Baltimore, Md. U.S.A Library of congress cataloging in publication data.

Heimer L (1968). Synaptic distribution of centripetal and centrifugal nerve fibres in the olfactory system of the rat. An experimental anatomical study. *J Anat* 103:413–432

Hilber P and Caston J, (2001). Motor skills and motor learning in Lurcher mutant mice during aging; *Neuroscience* 102 615–623

Hoglinger, G.U., Rizk, P., Muriel, M.P., Duyckaerts, C., Oertel, W.H., Caille, I., and Hirsch, E.C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat. Neurosci.* 7, 726–735.

Horstmann, E.(1954). Die faserglia des Selachiegchirns.Z. Zcllforsch. *Mikros. Anat.*, 39: 588 - 617.

Hubel, D.N., and Wiesel, T.N. (1962). Receptive fields, binocular interactions and functional architecture in the cat's visual cortex. *Journal of Physiology*, 160, 106–154.

Hue O A, Seynnes O, Ledrole D, Colson SS and Bernard PL, (2004). Effects of a physical activity program on postural stability inolder people; *Aging Clin. Exp. Res.* 16 356–362

Ibe, C.S., B.I. Onyeanusi, J.O. Hambolu and J.O. Ayo, (2010a). Sexual dimorphism in the whole brain and brainstem morphometry in the African giant pouched rat (*Cricetomys gambianus*,Waterhouse 1840). *Folia Morphol* (Warsz).May; 69(2):69-74.

Ibe, C.S., B.I. Onyeanusi, S.O. Salami and I.E. Ajayi, (2010b). Adaptive Morphology of the Mystacial Vibrissae in the African Giant Pouched Rat (*Cricetomys gambianus*,Waterhouse-1840) *J. Vet. Anat.*3(2): 35–46.

Ibe, C.S., B.I. Onyeanusi, S.O. Salami, I.E. Ajayi and J.O. Nzalak, (2010c). On the structure of the spleen in the African giant rat (*Cricetomys gambianus*, Waterhouse 1840). *Veterinary Research* 3(4):70-74.

Ibe, C.S., B.I. Onyeanusi, J.O. Hambolu and J.O. Ayo, (2011a). Nuclear architecture in the medulla oblongata of the adult African giant pouched rat (*Cricetomys gambianus*, Waterhouse - 1840). *Int. J. Morphol.*, 29(2):382-388.

Ibe, C.S., S.O. Salami and B.I. Onyeanusi, (2011b). Macroscopic anatomy of the lower respiratory system in a nocturnal burrowing rodent: African giant pouched rat (*Cricetomys gambianus*, Waterhouse 1840).*Anat Histol Embryol.* 40 (2):112-9

Ibe, C.S., B.I. Onyeanusi, S.O. Salami and J.O. Nzalak, (2011c). Microscopic Anatomy of the Lower Respiratory System of the African Giant Pouched Rat (*Cricetomys gambianus*)Waterhouse 1840): *Int. J. Morphol.*, 29(1):27-33

Ibe, C.S., Onyeanusi, B.I. and Hambolu, J.O., (2014). 'Functional morphology of the brain of the African giant pouched rat (*Cricetomys gambianus* Waterhouse, 1840) *Onderstepoort Journal of Veterinary Research* 81(1):7

Igbokwe, C.O. and I.C. Nwaogu, (2009). Histological studies of the vomeronasal organ of African Giant Rat (*Cricetomys gambianus*, Waterhouse), *Animal Research International* 6(2): 1003 – 1008.

Ihunwo, A.O. and Pillay, S. (2007). Neurogenic sites in the Adult Mammalian central nervous system.*Research Journal of Biological Sciences.* 2(2): 170- 177.

Imosemi, (2011).Studies on the role of Calotropis procera leaf extract and standard anti-oxidants in phenytoin induced neurotoxicity in developing cerebellum of wistar rats, *PhD thesis*, Department of Anatomy, College of Medicine, Lagos State University.

Jacobs, B., J. Lubs, M. Hannan, (2011). Neuronal morphology in the African Elephant (*Loxodonta africana*) neocortex.*Brain Struct.Funct.*215: 273–298. doi: 10.1007/s00429-010-0288-3.

Jacobsen M (1991). Developmental neurobiology. New York: Plenum.

Jalenques I, Burette A, Albuisson E and Romand R (1997). Age related changes in GFAP immunoreactive astrocytes in the rat ventral cochlear nucleus; *Hearing Res.* 107 113–124

Jang, M.-H., Song, H., and Ming, G.l. (2007). Regulation of Adult Neurogenesis by Neurotransmitters. In Adult Neurogenesis,

Gage F.H., G. Kempermann, and H. Song, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp397–423.

Jansa and Weksler M. (2004). Phylogeny of muroid rodents: relationships within and among major lineages as determined by IRBP gene sequences. *Mol. Phyl. Evol.*31(1): 256–276.

Jessberger, S., and Kempermann, G. (2003). Adult-born hippocampal neurons mature into activity-dependent responsiveness. *Eur. J. Neurosci*. 18, 2707–2712.

Jin, K., Sun, Y., Xie, L., Batteur, S., Mao, X.O., Smelick, C., Logvinova, A., and Greenberg, D.A. (2003). Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. *Aging Cell* 2, 175–183.

Johansson, C.B., Svensson, M., Wallstedt, L., Janson, A.M., and Frisen, J. (1999). Neural stem cells in the adult human brain. *Exp. Cell Res*. 253, 733–736.

Ka´lma´n M, and Hajos F (1989). Distribution of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the rat brain. *Exp Brain Res* 78:147–163.

Kawauchi, T. and M. Hoshino. (2008). Molecular pathways regulating cytoskeletal organization and morphological changes in migrating neurons. *Dev. Neurosci.* 30: 36–46.

Kee, N., Teixeira, C.M., Wang, A.H., and Frankland, P.W. (2007). Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat. Neurosci.* 10, 355–362.

Kempermann, G., and Gage, F.H. (2002). Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. *Eur. J. Neurosci.* 16, 129–136.

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., Gage, F. (2003). Early determination and long-term persistence of adult generated new neurons in the hippocampus of mice. *Development* 130: 391-399.

Kempermann, G., H. Georg Kuhn, and Fred H.G. (1997).Genetic Influence on neuron genesis in the dentate gyrus of adult mice. *Proc Natl Acad Sci USA.* 10409- 10414.

Kimelberg HK. (2004). The problem of astrocyte identity.*Neurochem Int.* 45:191–202.

Kingdon, J. (1989). East African mammals. London, New York: academic press.

Kingdon, J. (1997). The Kingdon Field Guide to African Mammals. Academic Press Limited, London.

Kirchhoff F, Dringen R and Giaume C (2001). Pathways of neuron–astrocyte interactions and their possible role in neuroprotection;*Eur. Arch. Psychiatry Clin.Neurosci.*251 159–169

Klempin F, Kronenberg G, Cheung G, Kettenmann H, Kempermann G (2011). Properties of doublecortin-(DCX)-expressing cells in the piriform cortex compared to the neurogenic dentate gyrus of adult mice. *PLoS One* 6(10):e25760. doi:10.1371/journal.pone.0025760

Klempin, F., and Kempermann, G. (2007). Adult hippocampal neurogenesis and aging. *Eur. Arch. Psychiatry Clin. Neurosci.* 257, 271–280.

Knight, M. (1988). Thermoregulation in the largest African cricetid, the giant rat Cricetomys gambianus. *Comparative Biochemistry and Physiology A-Physiology*, 89(4): 705-708.

Knight, M.H. and A. Knight-Eloff, (1987). Digestive tract of the African giant rat, *Cricetomys gambianus. J. Zool., Lond.*213:7-22.

KokoevaMV, Yin H, Flier JS (2007). Evidence for constitutive neural cell proliferation in the adult murine hypothalamus. *J Comp Neurol* 505:209–220

Komuro, H. and Rakic P. (1992). Selective role of N-type calcium channels in neuronal migration. *Science* 257: 806–809.

Korth, W. M. and Emry, R. J. (1991). The skull of *Cedromus* and a review of the Cedromurinae (Rodentia, Sciuridae). *Journal of Paleontology,* 65:984-994.

Kosaka T,and Kosaka K (2009). Olfactory bulb anatomy. In: Squire LR(ed) Encyclopedia of neuroscience, 7th edn. Oxford UniversityPress, Oxford, pp 59–69

Kratskin IL, Belluzzi O (2003). Anatomy and neurochemistry of the olfactory bulb. In: Doty RL (ed) Handbook of olfaction and gustation, 2nd edn. Marcel Dekker, New York, pp 235–276

Kreiner, G. (1934). Bulbus olfactorius der weissen ratte (topographie und myeloarchitektonik). *Z. Anat. Entwicklungsgesch.* 102: 232-245. 1934.

Krieg, W. J. S. (1946). Connection of the cerebral cortex: 1. The albino rat, topography of the cortical areas. *J. Comp. Neurol*. 84: 221-275. 1946.

Kuhn H.G., Dickson-Anson H., Gage F.H. (1996). Neurogenesis in the dentate of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neursci* 16:2027-2033.

Kuhn, H.G., Winkler, J., Kempermann, G., Thal, L.J., and Gage, F.H. (1997). Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J. Neurosci.* 17, 5820–5829.

Landry CF, GO Ivy, and IR Brown (1990). Developmental Expression of Glial Fibrillary Acidic Protein mRNA in the Rat Brain Analyzed by In Situ Hybridization, *Journal of Neuroscience Research* 25: 194-203

Laplagne, D.A., Esposito, M.S., Piatti, V.C., Morgenstern, N.A., Zhao, C., van Praag, H., Gage, F.H., and Schinder, A.F. (2006). Functional convergence of neurons generated in the developing and adult hippocampus. PLoS Biol. 4, e409.

Laplagne, D.A., Kamienkowski, J.E., Esposito, M.S., Piatti, V.C., Zhao, C., Gage, F.H., and Schinder, A.F. (2007). Similar GABAergic inputs in dentate granule cells born during embryonic and adult neurogenesis. *Eur. J. Neurosci.* 25: 2973–2981.

Lavocat, R. (1973). Les rongeurs du Miocene d'Afrique Orientale. *Mémoires et Travaux de l'Ecole Pratique des Hautes Etudes, Institut de Montpellier* 1: 1–284.

Lavocat, R. (1978). Rodentia and Lagomorpha. In: Maglio VJ, Cooke HBS, eds. *Evolution of African mammals*. Cambridge, MA: Harvard University Press, 69–89.

Leibnitz, L. (1972). Untersuchungen zur Optimierung der Gewichts- und Volumenanderungen von Hirnen wahrend der Fixierung.Dehydrierung und Aufhellung, sowie uber Ruckschlusse vom Gewicht des behandelten auf das Volumen des frischen Gehirns.*J. Hirnforsch.*13: 321- 329, 1972.

Leuner, B., Gould, E., and Shors, T.J. (2006). Is there a link between adult neurogenesis and learning? *Hippocampus* 16, 216–224.

Levinger, J.M. (1971). The cerebral ventricles of the rat. *J. Anat*. 108: 447- 451, 1971.

Lewis SA, and Cowan NJ (1985). Temporal expression of mouse glial fibrillary acidic protein mRNA studied by a rapid *in situ* hybridization procedure. *J Neurochem* 45:913-919

Lie, D.C., Colamarino, S.A., Song, H.J., Desire, L., Mira, H., Consiglio, A., Lein, E.S., Jessberger, S., Lansford, H., Dearie, A.R., et al. (2005). Wnt signaling regulates adult hippocampal neurogenesis. *Nature* 437, 1370–1375.

Lim, D.A., Tramontin, A.D., Trevejo, J.M., Herrera, D.G., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (2000). Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 28, 713–726.

Lindow, M. (2001). The Landmines- Sniffing Rats of the Mozambique. Time Magazine. Retrieved on 2008-06-23

Liu, Karen Jialing, Solway,Robert O. Messing,and Frank R. Sharp (2008).Increased Neurogenesis in the Dentate Gyrus After Transient Global Ischemia in Gerbils, The *Journal of Neuroscience*, *18*(19):7768–7778

Lledo P, Alonso M, Grubb S (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nat Rev Neurosci* 7:179–193.

Lledo, P.M., and Saghatelyan, A. (2005). Integrating new neurons into the adult olfactory bulb: joining the network, life-death decisions, and the effects of sensory experience. *Trends Neurosci*. 28, 248–254.

Lumsden, A. (2004). Segmentation and compartition in the early avian hindbrain.*Mech. Dev*. 121: 1081–1088.

Luzzatiet F, Bonfanti L, Fasolo A, Peretto P (2009). DCX and PSANCAM expression identifies a population of neurons preferentially distributed in associative areas of different pallial derivatives and vertebrate species. *Cereb Cortex* 19:1028– 1041

Luzzatiet, F., De Marchis, S., Fasola, A., Panetto, P. (2007). Adult neurogenesis and local neuronal progenitors in the striatum.*Neurodegener. Dis*. 4(4):322-327.

Madsen, T.M., Kristjansen, P.E., Bolwig, T.G., and Wortwein, G. (2003). Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. *Neuroscience* 119, 635–642

Madekurozwa M.C., B.O. Oke and A.K. Akinloye, (2010). The immunohistochemical localization of desmin and smooth muscle actin in the ovary of the African giant rat (*Cricetomys gambianus*) during the oestrous cycle. *Anat. Hist. Embryol.*, 39(1): 81-86.

Mandairon, N., Sacquet, J., Garcia, S., Ravel, N., Jourdan, F., and Didier, A. (2006). Neurogenic correlates of an olfactory discrimination task in the adult olfactory bulb. *Eur. J. Neurosci.* 24, 3578–3588.

Marschner C (1970). Qualitative und quantitative untersuchungen am bulbus olfactorius des elefanten im vergleich mit dem des menschen und des schweines. Acta Anat 75:578–595

Maslov AY, Barone TA, Plunkett RJ, Pruitt SC (2004). Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J Neurosci* 24:1726–1733

Matsunami, H. and M. Takeichi. (1995). Fetal brain subdivisions defined by T- and E- cadherins expressions: Evidence for the role of cadherin activity in region-specific, cell-cell adhesion. *Dev. Biol.* 172: 466–478.

Matsuo N, Kawamoto S, Matsubara K, Okubo K (1998). Cloning and developmental expression of the murine homolog of doublecortin. *Biochem Biophys Res Commun* 252:571–576

Matthews, M. A.; *St.* Onge, M.F. and Falcane, C. L.(1979). An electron microscopic analysis of abnormal ependymal cell proliferation and development of sprouting axons following spinal cord transection in the rat. *ActaNeuropathol.*, 45: 27- 36.

McConnell, S. K. and C. E. Kaznowski. (1991). Cell cycle dependence of laminar determination in developing neocortex. *Science* 254: 282–285.

Merkle, F.T., Mirzadeh, Z., and Alvarez-Buylla, A. (2007). Mosaic organization of neural stem cells in the adult brain. *Science* 317, 381–384.

Meshi, D., Drew, M.R., Saxe, M., Ansorge, M.S., David, D., Santarelli, L., Malapani, C., Moore, H., and Hen, R. (2006). Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat. Neurosci.* 9, 729–731

Miller, F. D. and A. S. Gauthier (2007). Timing is everything: Making neurons versus glia in the developing cortex. *Neuron* 54: 357–369.

Mirescu, C., and Gould, E. (2006). Stress and adult neurogenesis. *Hippocampus* 16, 233–238.

Molofsky, A.V., Slutsky, S.G., Joseph, N.M., He, S., Pardal, R., Krishnamurthy, J., Sharpless, N.E., and Morrison, S.J. (2006). Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* 443, 448–452.

Mori K, Kishi K, Ojima H (1983). Distribution of dendrites of mitral, displaced mitral, tufted, and granule cells in the rabbit olfactory bulb. *J Comp Neurol* 219:339–355

Morrow, T., M. R. Song and A. Ghosh. (2001). Sequential specification of neurons and glia by developmentally regulated extracellular factors. *Development* 128: 3585–3594.

Moutairou K., N. Hayez, V. Pohl, G/ Pattyn and R. Pochet, (1996). Calbindin localization in African giant rat kidney (*Cricetomys gambianus*). *BiochimBiophysActa*, 11; 1313(3):187-93.

Mukerjee, B. and T. Rajan, (2006). Morphometric study of Seminal Vesicle of rat in Normal Health and Stress Conditions. *J. Anat. Soc. India* 55 (1): 31-36.

Musser GG and MD.Carleton (2005). Family Muridae. In: Wilson DE, Reeder DM, eds. *Mammal species of the world:a taxonomic and geographic reference*. Baltimore: The Johns Hopkins University Press, 745–752.

Myers, P., R. Espinosa, C. S. Parr, T. Jones, G. S. Hammond, and T. A. Dewey. (2006). The Animal Diversity Web (online). Accessed July 21, 2008 at http://animaldiversity.org. Sponsored in part by the Interagency Education Research Initiative, the Homeland Foundation and the University of Michigan Museum of Zoology. The Regents of the University of Michigan and its licensors.

Nacher J, Daniel R. Rosell, Gregori Alonso-Llosa and Bruce S. McEwen (2001). NMDA receptor antagonist treatment induces a long-lasting increase in the number of proliferating cells, PSA-NCAM-immunoreactive granule neurons and radial glia in the adult rat dentate gyrus, *European Journal of Neuroscience*, 13: 512-520

Nacher, J., and McEwen, B.S. (2006). The role of N-methyl-D-asparate receptors in neurogenesis. *Hippocampus* 16, 267–270.

Nag, S. (2011). Morphology and properties of astrocytes, *Methods Mol Biol*. 686:69-100. doi: 10.1007/978-1-60761-938-3_3

Nancy Ann Oberheim, Steven A. Goldman, and Maiken Nedergaard, (2012). Heterogeneity of Astrocytic Form and Function, *Methods Mol Biol*.; 814: 23–45. doi:10.1007/978-1-61779-452-0_3.

Nandy K (1981). Morphological changes in the cerebellar cortex of aging *Macaca nemestrina*; *Neurobiol Aging*.2:61–64.

Ngwenya A, Patzke N, Ihunwo AO, Manger PR (2011). Organization and chemical neuroanatomy of the African elephant (Loxodonta africana) olfactory bulb. *Brain Struct Funct* 216:403–416

Nichols, D. H. (1981). Neural crest formation in the head of the mouse embryo as observed using a new histological technique. *J. Embryol. Exp. Morphol.* 64: 105–120.

Niquet J, Gillian A, Ben-Ari Y and Represa A (1996). Reactive glial cells express a vitronectin-like protein in the hippocampus ofepileptic rats; *Glia* 16 359–367

Nomura, T., M. Takahashi, Y. Hara and N. Osumi. (2008). Patterns of neurogenesis and amplitude of *Reelin* expression are essential for making a mammalian-type cortex. *PLoS One* 3: e1454.

Noorulla Mohamed, and Venugopala Rao B., (2014), Stratification of Spinal Ependyma in Rats in Response to Injury; *Research and Reviews:* Journal of Medical and Health Sciences RRJMHS, Volume 3;Issue 1

Nottebohm, F. (2004). The road we travelled: discovery, choreography, and significance of brain replaceable neurons. *Ann. N Y Acad. Sci.* 1016, 628–658.

Nowak R (1999): Walker's mammals of the world. The John Hopkins University Press, Baltimore and London. Vol. 2

Nowak, R.M., and J.L. Paradiso. (1983). Walker's Mammals of the world, 4th ed. Vol. II. Baltimore, Johns Hopkins Univ. Pr. 569-1362

Nssien MAS, Olayemi FO, Onwuka SK, Akin O. (2002).Comparison of some plasma biochemical parameters in two generations of African Giant Rat (*Cricetomys gambianus*, Waterhouse).*African Journal of Biomedical Research* 5: 63–67.

Nzalak JO, Byanet O, Salami SO, Umosen AD, Maidawa SM, Ali MN, Imam J (2008). Comparative morphometric studies of the cerebellum and forebrain of the african giant rat (AGR) (*Cricetomys gambianus* – Waterhouse) and that of the Grasscutter (*Thryonomys Swinderianus*). *J Animal Veter Adv*, 7: 1090-1092.

Nzalak, J.O., B.I. Onyeanusi, S.A.Ojo, A.A. Voh and C.S. Ibe, (2010). Gross anatomical histological and histochemical studies of the oesophagus of the African giant (*Cricetomys gambianus*, Waterhouse), Journal of Veterinary Anatomy 3(2):55-64.

Nzalak, J.O., J.O. Ayo, J.S. Neils, J.O. Okpara, B.I. Onyeanusi, A. Ghaji and S.A. Ojo, (2005). Morphometric studies of the cerebellum and forebrain of the African giant rat (*Cricetomys gambianus*, Waterhouse) *Trop. Vet.* 23;(3&4):87-92.

Ogata K and Kosaka T. (2002).Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* 113:221–233.

Oke, B.O. and Aire, T.A. (1989). The Bulbourethral (Cowper's) gland of the African giant rat (*Cricetomys gambianus*, Waterhouse). *Veterinaski.Archiv*.59:267-274.

Oke, B.O. and T.A. Aire, (1995). The prostate gland of the African giant rat (*Cricetomys gambianus*, Waterhouse). *Archives of Veterinary Medicine,* 65(4): 115-125.

Oke, B.O., (1985). Effect of season on the reproductive organs of the male African Giant Rat (*Cricetomys gambianus*, Waterhouse) in Ibadan, Nigeria. *African J. Ecology,* 23:67-70.

Oke, B.O., (1988). Some aspects of the reproductive biology of the male African giant rat (Cricetomys gambianus-Waterhouse). (Ph.D Thesis, University of Ibadan).

Oke, B.O., (1995). Some histochemical features of the epididymis of the African giant rat (*Cricetomys gambianus*, Waterhouse). Archives of Veterinary Medicine, 65(3): 69-76.

Oke, B.O., (1989). The ultrastructure of the epididymis of the African giant rat (*Cricetomys gambianus*, Waterhouse). *J. Anatomy, (London),* 165:75-89.

Oke, B.O., (1987). The structure of the epididymis of the African the giant rat (*Cricetomys gambianus*, Waterhouse). Histochemical and microstereological Studies. *J. Anatomy, (London),* 160: 9-19.

Oke, B.O.and Aire, T.A. (1990). Ultrastructural evidence for secretion in different zones of the caput epididymis of the African giant rat (*Cricetomys gambianus*,Waterhouse). *Archives of Veterinary Medicine*, 60 (4):207-212.

Oke, BO and Aire TA, (1996). The ampullary gland of the African giant rat *(Cricetomys gambianus*, Waterhouse). *Nig. Vet. J*. 1(1): 88-95.

Oke, BO and Aire TA, (1997). The epithelium of the vesicular gland of the African giant rat (*Cricetomys gambianus*, Waterhouse): Histology and Ultrastructure. *Afr. J. Med. & Med. Sci.* 26: 69-72.

Oke, O.A. and B.O. Oke, (1999). Vaginal Cytological changes during the Oestrous cycle of the Adult Female African Giant Rat (*Cricetomys gambianus*, Waterhouse). *Trop. Vet.* 17:169-180.

Oke, O.A., (2000). Haematological changes during the oestrus cycle of the African giant rat (*Cricetomys gambianus*, Waterhouse) *Tropical* Vet. 18: 202-206.

Olariu, A., Yamada, K., and Nabeshima, T. (2005). Amyloid pathology and protein kinase C (PKC): possible therapeutics effects of PKC activators. *J. Pharmacol. Sci*. 97, 1–5.

Olayemi, A. and A. Akinpelu, (2008). Morphometric characterization of the Giant Pouched Rat (*Cricetomys gambianus,*Waterhouse 1840) in the forest zone of South Western Nigeria, Mammalia, 72(3):229-236

Olayemi A, V Nicolas, J Hulselmans A.D. Missoup, E Fichet-Calvet, D. Amundala, A. Dudu, T. Dierckx, W. Wendelen, H. Leirs and E. Verheyen (2012). Taxonomy of the African giant pouched rats (Nesomyidae: *Cricetomys*): molecular and craniometric evidence support an unexpected high species diversity *Zoological Journal of the Linnean Society*,165, 700–719..

Olayemi, F.O., O.A. Oke, J.O Oyewale and A.O. Ogunsanmi, (2001). The effect of season on the blood profile of the African giant rat (*Cricetomys gambianus,*Waterhouse). *Israel Journal of Veterinary Medicine,* 56:147-150.

Olopade, J. O. & Onwuka, S. K. (2002). Preliminary Morphometric Investigation of the brain of Red Sokoto (maradi) goat. *Trop. Vet.,* 20(2):80-4,

Olowo-okorun, M.O., (1979). Some aspects of the physiology of the domesticated African giant rat. In Savannah Woodland management.Recent progress in African studies. Ajayi,S.S and Holstead, L.B. (Eds). *Taylor and Francis ltd, London,* 142-150.

Olson, A.K., Eadie, B.D., Ernst, C., and Christie, B.R. (2006). Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus*, 16: 250–260.

Olude, M.A., (2009a). Craniomorphometric measurements of the skull of the African giant rat (*Cricetomys gambianus*, Waterhouse). *Masters Dissertation* in the department of Veterinary Anatomy, University of Ibadan.

Olude M.A., Olopade J.O., Fatola I.O., & Onwuka S.K.. (2009b). Some aspects of the neurocraniometry of the African giant rat (*Cricetomys gambianus*, Waterhouse). *Folia Morphol*, 68: 224-227

Olude, M.A., J.O. Olopade and O.A. Mustapha, (2009c). Macro-anatomical investigations of the skeletons of the African giant rat (*Cricetomys gambianus Waterhouse*): *Pelvic limb. Eur. J. Anat.*13: 127-131.

Olude, M.A. and J.O. Olopade, (2010a). Morphometric Studies of the Axial Skeleton of the African Giant Rat (*Cricetomys gambianus,* Water-house) Part (1): Skull Typology. *J. Vet. Anat. 3: 1-12.*

Olude, M.A., J.O. Olopade, A.K. Akinloye and O.A. Mustapha, (2010b). Macro-anatomical investigations of the skeletons of the African giant rat (Cricetomys gambianus Waterhouse 1840) II: Fore limb *Eur J Anat*.14 (1): 19-23.

Olude, M.A., J.O. Olopade, O.O Igado, O.A. Mustapha and A.K. Akinloye, (2011). Some aspects of the Orbital and Ocular Morphometry of the African Giant Rat (*Cricetomys gambianus)*. *Vet. Anat.*, 4 (1): 11 – 18.

Olude, M.A., Olopade, J.O., Mustapha A.O., and Ogunbunmi T.K. (2013). The vertebral column, ribs and sternum of African giant rat (*Cricetomys gambianus* Waterhouse 1840). *The Scientific World Journal* ISSN 1537-744X dx.doi.org/10.1155/2013/973537

Olude M. A, Ogunbunmi T.K, Olopade J.O. and Ihunwo A.O., (2014a). The olfactory bulb structure of African giant rat (Cricetomys gambianus, Waterhouse 1840) I: cytoarchitecture, *Anat Sci Int* 89 (4): 224-231

Olude, M.A., Olopade, J.O. and Ihunwo A.O, (2014b). Adult neurogenesis in the African Giant Rat (*Cricetomys gambianus*, Waterhouse) *Metabollic Brain Disease*, 29:857–866

Onyeanusi, B.I., A.A. Adeniyi, J.O. Ayo and J.O. Nzalak, (2007), Morphometric Studies on the Kidneys of the African Giant Rat (*Cricetomys gambianus*, Waterhouse) *Journal of Animal and Veterinary Advances*, 6(11):1273-1276.

Onyeanusi, B.I., A.A. Adeniyi, J.O. Ayo, C.S. Ibe and C.G. Onyeanusi, (2009). Comparative Study on the Urinary System of the African Giant Rat (*Cricetomys gambianus*) and the Wistar Rat. Pakistan Journal of Nutrition, *Pak. J. Nutr.*, 8(7):1043-7

Overstreet Wadiche, L., Bromberg, D.A., Bensen, A.L., and Westbrook, G.L. (2005). GABAergic signaling to newborn neurons in dentate gyrus. *J. Neurophysiol.* 94: 4528–4532.

Oyerekua, M.A. and Ketiku, A.O. (2010). The nutrient composition of the AGR. *Advanced journal of food science and technology* 2(6):318-324

Oyewale, J., F.O. Olayemi, O.A. Oke (1998). Haematology of wild African giant rat (*Cricetomys gambianus*), *Vet. Arhiv* 68:91-99

Pakkenberg B, Gundersen HJG (1997). Neocortical neuron number in humans: effect of sex and age. *J Comp Neurol* 384:312–320

Palmer, T.D., Willhoite, A.R., and Gage, F.H. (2000). Vascular niche for adult hippocampal neurogenesis. *J. Comp. Neurol.* 425: 479–494..

Patzke N, Kaswera C, Gilissen E, Ihunwo AO, Manger PR (2013). Adult neurogenesis in a giant Otter shrew (Potamogale velox). *J Neurosci* 238:270–279

Pehar M, Cassina P, Vargas MR, Castellanos R, Viera L, Beckman JS, Estevez AG, Barbeito L. (2004). Astrocytic production of nerve growth factor in motor neuron apoptosis: implications for a myotrophic lateral sclerosis. *Journal of Neurochemistry*.89: 464–473.

Peretto P, Merighi A, Fasolo A, Bonfanti L (1997). Glial tubes in the rostral migratory stream of the adult rat. *Brain Res Bull*, 42(1):9–21.

Perry, N.D, B. Hanson, W. Hobgood, R.L Lopez, C.R. Okraska, K. Karem, I.K. Damon and D.S. Carroll, (2006). New invasive species in southern Florida: Gambian rat (*Cricetomys gambianus*). *Journal of Mammalogy,* 87: 262–264.

Peterson T.A., M. Papes, M.G. Reynolds, N.D. Perry, B. Hanson, R.L. Regnery, C.L. Hutson, B. Muizniek, I.K. Damon and D.S. Carroll, (2006). Native range ecology and invasive potential of *Cricetomys* in North America. *Journal of Mammalogy,* 87: 427–432.

Petreanu, L., and Alvarez-Buylla, A. (2002). Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. *J. Neurosci.*, 22: 6106–6113.

Petter, F. (1966). Affinités des genres *Beamys*, *Saccostomus* et*Cricetomys* (Rongeurs, Cricetomyinae). *Annales MuséeRoyal d l'Afrique Centrale. Sciences Zoologiques* 144: 13–25.

Piechl, L. and K. Moutairou, (1998). Absence of wavelength sensitive cones in the retinae of seals (Carnivora) and African giant rat- *European Journal of Neuroscience* 10: 2586-2594.

Porras-Garcia E, Cendelin J, Dominguez-del-Toro E, Vozeh F andDelgado-Garcia J M (2005). Purkinje cell loss affects differentially the execution, acquisition and prepulse inhibition of skeletal and facial motor responses in Lurcher mice; *Eur J Neurosci.* 21: 979–988

Price JL, and Powell TPS (1970). The morphology of the granule cells of the olfactory bulb. *J Cell Sci* 9:91–123

Prochiantz A, Mallat M. (1988) Astrocyte diversity. *Annals of the New York Academy of Science.* 540:52–63.

Purkinje, J.E.(1836). Veber Filmmerbewegungen in Gehrim Muller. *Arch. Anat. Physiol.* 289: 66 - 71.

Qian, X., Q. Shen, S. K. Goderie, W.He, A. Capela, A. A. Davis and S. Temple. (2000). Timing of CNS cell generation: A programmed sequence of neuron and glial cell production from isolated murine cortical stem cells. *Neuron* 28: 69–80.

Quenum, A and R. Camain, (1959). Histochemical study of the adrenal cortex of *Cricetomys gambianus*. *C R SeancesSocBiol Fil.*153:697-701.

Quenu m A., (1961). The pituitary cellular types and their localizations in *Cricetomys gambianus.C R SeancesSocBiolFil.,* 155:396-398.

Quenum, A. and R. Camain, (1962). Castration and the adrenal cortex in *Cricetomys gambianus.CR SeancesSocBiolFil.* 156:317-24.

Quenum,A., (1962a). Genital hormones and histochemical changes of the adrenal cortex of *Cricetomys gambianus*. *C R SeancesSocBiolFil*.156:722-5.

Quenum A., (1962b) Inhibitory action of testosterone on compensatory adrenal hypertrophy: study in the male *Cricetomys gambianus*. *C R SeancesSocBiolFil*.156:313-7.

Quenum A.,(1962c) Histo-functional significance of several pituitary cell types in *Cricetomys gambianus.C R SeancesSocBiolFil.* 156:712-6.

Raber, J., Rola, R., LeFevour, A., Morhardt, D., Curley, J., Mizumatsu, S., VandenBerg, S.R., and Fike, J.R. (2004). Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat. Res.* 162, 39–47.

Rakic, P. (1972). Mode of cell migration to superficial layers of fetal monkey neocortex.*J. Comp. Neurol.* 145: 61–83.

Rakic P. (1974). Radial cells: scaffolding for brain construction. In: *Neuroglia*, edited by Kettenmann H and Ransom BR. Oxford, UK: Oxford Univ. Press,. 746–762.

Rakic, P. (2002). Neurogenesis in adult primate neocortex: an evaluation of the evidence. *Nat. Rev. Neurosci*. 3: 65–71.

Rakic, P. (1975). Cell migration and neuronal ectopias in the brain. *In* D. Bergsma (ed.), *Morphogenesis and Malformations of Face and Brain*.Birth Defects Original Article Series, 11: 7. *Alan R. Liss*, New York, pp. 95–129.

Rakic, P. and P. S. Goldman. (1981). Development and modifiability of the cerebral cortex. *Neurosci.Rev.* 20: 429–611.

Rakic, P. and R. L. Sidman. (1973). Organization of cerebellar cortex secondary to deficit of granule cells in *weaver* mutant mice. *J. Comp. Neurol.* 152: 133–162.

Rakic, P. (1995). Corticogenesis in human and non-human primates. In M. S. Gazzaniga (Ed.), The cognitive neurosciences Cambridge MA: MIT Press. pp. 127–145.

Rakic, P., A. E. Ayoub, J. J. Breunig and M. H. Dominguez (2009). Decision by division: Making cortical maps. *Trends Neurosci*. 32: 291–301.

Ramirez-Castillejo, C., Sanchez-Sanchez, F., Andreu-Agullo, C., Ferron, S.R., Aroca-Aguilar, J.D., Sanchez, P., Mira, H., Escribano, J., and Farinas, I. (2006). Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat. Neurosci.* 9: 331–339.

Reichenbach A, Wolburg H. (2005). Astrocytes and ependymal glia. In: Kettenmann H, Ransom BR,editors. Neuroglia.Oxford University Press; pp. 19–35.

Reid, C. B., I. Liang and C. Walsh. (1995). Systematic widespread clonal organization in cerebral cortex. *Neuron* 15: 299–310.

Reig, O.A. (1980). A new fossil genus of South American cricetid rodents allied to *Wiedomys*, with an assessment of the Sigmodontinae. *Journal of Zoology, London* 192: 257–281.

Reig, O.A. (1981). Teorio del origen y desarrollo de la fauna de mamíferos de America del Sur. Publicadas por el Museo Municipal de Ciencias Naturales 'Lorenzo Scaglia'. *Monografie Naturae* 1: 182.

Remahl S, Hildebrand C (1985). Myelinated non-axonal elements in the feline olfactory bulb lack sites with a nodal structural differentiation. *Brain Res* 325:1–11.

Renovell A, Giner J and Portoles M (1996). Loss of granule neurons in the aging human cerebellar cortex; *Int. J. Dev. Biol.(Suppl.)*1 193–194.

Reynolds R AND Wilkin GP. (1991). Oligodendroglial progenitor cells but not oligodendroglia divide during normal development of the rat. *J Neurocytol* 20: 216–224

Rio Hortega DP, (1928). Tercera aportacion al conocimiento morfologico e interpretacion funcional de la oligodendroglia.*Memor Real SocEsp Hist Nat* 14: 5–122.

Roberts, A. (1951). *The mammals of South Africa*. Johannesburg, South Africa.

Rola, R., Raber, J., Rizk, A., Otsuka, S., VandenBerg, S.R., Morhardt, D.R., and Fike, J.R. (2004). Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. *Exp. Neurol.* 188, 316–330.

Rosevear, D.R. (1969). *The rodents of West Africa*. London: British Museum (Natural History).

Russel, M. S. (1979). *The brain size and intelligence: comparative perspective in brain behavior and evolution* (Oakly and Plotkin ed.). London, Associated Book Publishers, pp.127-53.

Ryan, J. (1989). Evolution of cheek pouches in African pouch rats (Rodentia: Cricetomyinae). *Journals of Mammology.* 70(2): 267-274.

Sabbatini M, Barili P, Bronzetti E, Zaccheo D and Amenta F, (1999). Age-related changes of glial fibrillary acidic protein immunoreactive astrocytes in the rat cerebellar cortex; *Mech. AgeingDev.*108: 165–172

Saghatelyan, A., Roux, P., Migliore, M., Rochefort, C., Desmaisons, D., Charneau, P., Shepherd, G.M., and Lledo, P.M. (2005). Activity-dependent adjustments of the inhibitory network in the olfactory bulb following early postnatal deprivation. *Neuron* 46, 103–116.

Salami, S.O., K.T. Onwuama, M.S. Maidawa, J. Imam and S.A. Ojo, (2011a). Morphological studies of the appendicular skeleton of the African giant pouched rat (*Cricetomys gambianus)* part (i) pectoral limb *Journal of Veterinary Medicine and Animal Health* 3(7): 82-87

Salami, S.O., K.T. Onwuama, O. Byanet, S.C. Ibe and S.A. Ojo (2011b) Morphological studies of the appendicular skeleton of the African giant pouched rat (*Cricetomys gambianus)* part (ii) pelvic limb *Journal of Veterinary Medicine and Animal Health*. 3(7): 88-93,

Sambo, S.J., Ibrahim N.D.G, Kazeem H.M., Adamu, S., Mammam, P.H. and Ali M.N. (2008). Veterinary hepatic capillariosis in African giant rats (*Cricetomys gambianus*, Waterhouse) around Zaria, Nigeria. *Research* 2(1):9-11

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301, 805–809.

Satoh. Y., K. Ono and K. Moutairou, (1994). Paneth cells of African giant rats (*Cricetomys gambianus*). *Acta Anat*., 151:49-53.

Saxe, M.D., Battaglia, F., Wang, J.W., Malleret, G., David, D.J., Monckton, J.E., Garcia, A.D., Sofroniew, M.V., Kandel, E.R., Santarelli, L., et al. (2006). Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc. Natl. Acad. Sci. USA* 103, 17501–17506

Saxe, M.D., Malleret, G., Vronskaya, S., Mendez, I., Garcia, A.D., Sofroniew, M.V., Kandel, E.R., and Hen, R. (2007). Paradoxical influence of hippocampal neurogenesis on working memory.*Proc. Natl. Acad. Sci. USA* 104, 4642–4646.

Schimke, R. and F. Ehrenbrand. (1968). Beitrage zur orthologie des corpus geniculatum laterale der ratte. *Anat. Anz*. 122: 75-92.

Schmalenbach C and Muller HW (1993). Astroglia–neuron interactions that promote long-term neuronal survival; *J. Chem.Neuroanat.*6: 229–237

Schmidt-Hieber, C., Jonas, P., and Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature 429, 184–187.

Schulz E.B., Thamke, H. Rehmer (1972). Qualitative und quantitative zytoarchitektonische untersuchungen zur Variabilitat des bulbus olfactorius der erwachsenen weissen laborratte (Rattus norvegicus formaalba).*J. Hirnforsch.* 19: 211-221.

Schwab ME and Schnell L. (1989). Region-specific appearance of myelin constituents in the developing rat spinal cord.*J Neurocytol* 18: 183–190,

Scott JW, McBride RL, Schneider SP (1980). The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. *J Comp Neurol* 194:519–534

Scotti Campos L (2003). Evidence for astrocyte heterogeneity: a distinct subpopulation of protoplasmic-like glial cells is detected in transgenic mice expressing Lmo1-lacZ. *Glia* 43:195–207

Selstam, G., I. Nilsson and M.O. Mattsson, (1993). Changes in the ovarian intermediate filament desmin during the luteal phase of the adult pseudo pregnant rat. *Acta Physiol. Scand.*147:123–129.

Shapiro LA, Ng KL, Kinyamu R, Whitaker- Azmitia P, Geisert EE, Blurton-Jones M, Zhou QY, Ribak CE (2007). Origin, migration and fate of newly generated neurons in the adult rodent piriform cortex. *Brain Struct Funct* 212:33–48

Sheets, B.S. (1989). Cranial anatomy of *Jaculus orientalis* (rodentia, dipoidea): new evidence for close relationship of dipodoid and muroid rodents. Submitted to the committee on undergraduate honors of Baruch College of the City University of New York in partial fulfillment of the requirements for the degree of Bachelor of art in biology with honors.

Shors, T.J. (2004). Memory traces of trace memories: neurogenesis, synaptogenesis and awareness. *Trends Neurosci.* 27, 250–256.

Shors, T.J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., and Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410, 372–376.

Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y., and Gould, E. (2002). Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 12, 578–584.

Simpson GG. (1945). The principles of classification and the classification of mammals. *Bulletin of the American Museum of Natural History* 85: 1–350.

Sisti, H.M., Glass, A.L., and Shors, T.J. (2007). Neurogenesis and the spacing effect: learning over time enhances memory and the survival of new neurons. *Learn. Mem.* 14: 368–375.

Smith, G. M. and Shine, D. (1992). Immuno-flouresence labeling of tight junctions in the rat brain and spinal cord. *Int. J. Dev. Neurosci*. 10: 387-393.

Smitka M, Abomaali N, Witt M et al (2009). Olfactory bulb ventricles as a frequent finding in magnetic resonance imagery studies of the olfactory system. *Neuroscience* 162:482–485

Snyder, J.S., Hong, N.S., McDonald, R.J., and Wojtowicz, J.M. (2005). A role for adult neurogenesis in spatial long-term memory. *Neuroscience* 130, 843–852.

Sodeinde O.A.and D.A. Soewu (1999). Pilot Study of the Traditional Medicine Trade in Nigeria With reference to wild faunal: *TRAFFIC Bulletin* 18 (1): 35-40

Sofroniew Michael V, Vinters Harry V (2010). Astrocytes: biology and pathology. *Acta neuropathologica* 119 (1): 7-35

Song, H., Stevens, C.F., and Gage, F.H. (2002). Astroglia induce neurogenesis from adult neural stem cells. *Nature* 417, 39–44.

Stanka, P. (1963). Uber das Subcommissuralorgan bei Schwein und Ratte.*Z. Mikrosk. Anat. Forsch*. 69: 395- 409

Stephan, H. (1954). Vergeichend-anatomische Untersuchungen an Hirnen von Wild-und Haustieren; II. Die Oberflachen des Allocortex bei Wild- und Hausform von *Epimys norvegicus. Morphol.Jahrb*. 93: 425-471

Steppan, S. J., R. A. Adkins, and J. Anderson (2004). Phylogeny and divergence date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. Systematic Biology, 53:533-553.

Suh, H., Consiglio, A., Ray, J., Sawai, T., D'Amour, K.A., and Gage, F.H. (2007). In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. *Cell Stem Cell* 1, 515–528.

Sulkowski G, Bubko I, Struzynska L, Januszewski S, Walski Mand Rafalowska U (2002). Astrocytic response in the rodent model of global cerebral ischemia and during reperfusion; *Exp. Toxicol. Pathol.* 54 31–38

Takahashi M, Arai Y, Kurosawa H, Sueyoshi N, Shirai S. (2003). Ependymal cell reactions in spinal cord segments after compression injury in adult rat. *J Neuropathol Exp Neurol*. 62(2):185-94.

Tardy M, Fages C, Riol H, LePrince G, Rataboul P, Charriere-Bertrand C, Nunez J (1989). Developmental expression of the glial fibrillary acidic protein mRNA in the central nervous system and in cultured astrocytes. *J Neurochem* 52: 162-1 67

Tashiro, A., Makino, H., and Gage, F.H. (2007). Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. *J. Neurosci.* 27, 3252–3259.

Tashiro, A., Sandler, V.M., Toni, N., Zhao, C., and Gage, F.H. (2006). NMDAreceptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* 442, 929–933.

Thomas O. (1904). On the Genera of Rodents, an attempt to bring up to date the current arrangement of the Order. *Proceedings of the Zoological Society, London* 1: 1012–1028.

Tokioka, T. (1973). The arterial system of the Spinal cord in the rat. Okajimas Folia Anat. Jap. 50: 133-182.

Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H., and Gage, F.H. (2007). Synapse formation on neurons born in the adult hippocampus. Nat. Neurosci. 10, 727–734.

Tozuka, Y., Fukuda, S., Namba, T., Seki, T., and Hisatsune, T. (2005). GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* 47, 803–815.

Tullberg, T. (1899). Ueber das system der Nagetiere: Eine phylogenetische Studie. *Nova Acta Reg. Soc. Sci. Upsala Ser.* 3 (18): 1-514

Turner, D. L. and C. L. Cepko. (1987). A common progenitor for neurons and glia persists in rat retina late in development. *Nature* 328: 131-136.

Valerius, K.P., (1996) Size-dependent morphology of the conductive bronchial tree in four species of myomorph rodents.*J Morphol.* 230(3):291-7.

Vallace MC, Tator CH, Lewis AJ, (1983). Regenerative activity in the spinal cord after acute compression injury in rats. *Surg Forum.*34:515-517.

Van Praag, H., Shubert, T., Zhao, C., and Gage, F.H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *J. Neurosci.* 25, 8680–8685.

Van der Knaap MS AND Valk J. (1995). Myelination and retarded myelination. In: Magnetic Resonance of Myelin, *Myelination and Myelin Disorders.* Berlin: Springer, p. 31–57

Vaquero J, Ramiro MJ, Oya S, Cabezundo JM, (1981). Ependymal reaction after experimental spinal cord injury. *Acta Neurochi.*55:295-302.

Vaughan, T.A., J.M. Ryan, and N.J. Czaplewski (2000). *Mammalogy*, 4th ed. New York, Saunders Coll. Publ. 565 pp.

Vaz Ferrerira, A. (1951). Cortical areas of albino rat studied by silver impregnation.*J. Comp. Neurol.* 95: 177-243.

Verhagen R., C. Cox, R. Machang'u, B. Weetjens and M.Billet (2003). Preliminary results on the use of Cricetomys ratsas indicators of buried explosives in field conditions. In:*Mine detection dogs: training operations and odour detection*.Geneva: Geneva International Centre for HumanitarianDemining, 175–193.

Volkmann, W. (1926). Vergleichende Untersuchunger an der Rinde der "motorischen" und "Sehregion" von Nagetieren. *Anat. Anz.* 61 (suppl.) 234-243

Wahlert, J.H., S.L. Sawitzke and M.E. Holden (1993). Cranial anatomy and relationships of dormice (Rodentia, Myoxidae), *Amer. Mus. Novitaes* 3016: 32 pp

Waibl, H. (1973). Zur Topographie der Medulla spinalis der Albinoratte (Rattus norvegicus) *Adv. Anat. Embryol. Cell Biol.* 47

Wallace, V. A. (1999). Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. *Curr. Biol.* 9: 445–448.

Wallraff A, Odermatt B, Willecke K, Steinhauser C, (2004). Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. *Glia.*48:36–43.

Walsh, C. and C. L. Cepko. (1988). Clonally related cortical cells show several migration patterns. *Science* 241: 1342–1345.

Wang, X., J. W. Tsai, J. H. Imai, W. N. Lian, R. B. Vallee and S. H. Shi. (2009). Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. *Nature* 461: 947–955.

Wang Xiaohong, Tetsuya Imura, Michael V. Sofroniew and Shinji Fushiki, (2011). Loss of adenomatous polyposis coli in Bergmann glia disrupts their unique architecture and leads to cell non-autonomous neurodegeneration of cerebellar Purkinje neurons, *Glia* ; 59(6): 857–868

Weetjens BJ, GF Mgode, RS Machang'u, R Kazwala, G Mfinanga, F Lwilla, C Cox, M Jubitana, H Kanyagha, R Mtandu, A Kahwa, J Mwessongo, G Makingi, S Mfaume, J. van Steenberge, NW Beyene, M Billet, R Verhagen. (2009). African pouched rats for the detection of pulmonary tuberculosis. *International Journal of Tuberculosisand Lung Disease* 13: 737–743.

Weindl, A. (1965). Zur morphologie und histochemie von subfornicalorgan.Organon vasculosum laminae terminalis und area postrema bei kaninchen und ratte. Z. Zellforsch. *Mikrosk. Anat.* 67: 740-775. 1965.

Weinstein, C.S., S. Weinstein and Drozdenko, (1992). "The challenge of bio-detection for persons carrying explosive detection". In S.M. khan (ed) Proceedings First International Symposium on explosive detection technology: 759-769.

Welker,W.I. &M. Carlson. (1976). Somatic sensory cortex of hyrax (*Procavia*). *Brain Behav.Evolut.*13: 294–301.

West, J. R., (1995). The cerebellum: In Neuroscience in Medicine, edited by Conn, P.M., J.B. Lippincott company, Philadelphia: chapter 12: 213- 225.

Westergaard, E.(1969). The cerebral ventricles of the rat during growth.*Acta Anat. (Basel)* 74: 405- 423

White, L., Jr. (1965). Olfactory bulb projections of the rat.*Anat. Rec.* 152: 465-480.

Wilkin GP, Marriott DR, Cholewinski AJ (1990). Astrocyte heterogeneity. TINS 13: 43-46.

Winocur, G., Wojtowicz, J.M., Sekeres, M., Snyder, J.S., and Wang, S. (2006). Inhibition of neurogenesis interferes with hippocampus-dependent memory function. *Hippocampus* 16, 296–304.

Wislocki,GB(1932). The cytology of the cerebrospinal pathway. In: Special cytology, E.V.Cowdry ed.PB. HoeberInc., New York, pp: 1485 - 1521.

Wu Y, Zhang A Q and Yew D T (2005). Age related changes ofvarious markers of astrocytes in senescence-accelerated mice hippocampus; *Neurochem. Int.* 46 565–574

Wunscher, W., W. Schober, and L. Werner. (1965). *Architektonischer Atlas vom Hirstamm der Ratte.* Leipzip S. Hirzel-Verlag,

Xiong K, Luo DW, Patrylo PR, Luo XG, Struble RG, Clough RW, Yan XX (2008). Doublecortin- expressing cells are present in layer II across the adult guinea pig cerebral cortex: partial colocalization with mature interneuron markers. *Exp Neurol* 211:271–282

Yakovlev PI and Lecours AR. The myelinogenic cycles of regional maturation of the brain (1966). In: *Regional Development of the Brain in Early Life*, edited by Minkovski A. Oxford, UK: Blackwell p.3–70

Zecevic N, Rakic P, (2001). Development of layer I neurons in the primate cerebral cortex. J Neurosci; 21:5607–5619.

Zemman, W. and Innes, J.R.M. Craigee's (1963). Neuroanatomy of the rat. New York Academic Press,

Zhang C, Hua T, Zhu Z and Luo X, (2006). Age-related changes of structures in cerebellar cortex of cat; *J. Biosci*.31 55–60

Zhang SC, (2001). Defining glial cells during CNS development.*Nature Reviews Neuroscience*, 2:840–843.

Zhang, C.-L., Zou, Y., He, W., Gage, F.H., and Evans, R.M. (2008). A role for adult TLX-positive neural stem cells in *Learning and Behaviour.Nature*. Published online January 30, 2008.10.1038/nature06562.

Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., and Gage, F.H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J. Neurosci.* 26, 3–11.