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Gliogenesis in the Male Japanese Quail (Coturnix japonica) Brain

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Gliogenesis in the Male Japanese Quail *(Coturnix japonica)* Brain



SHEHU, Asmau Muhammad

1789432

This thesis is submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

SCHOOL OF ANATOMICAL SCIENCES

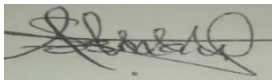
UNIVERSITY OF THE WITWATERSRAND

JOHANNESBURG

OCTOBER 2025

Declaration

I, Shehu Asmau Muhammad, declare that this thesis is my original and unaided work. It is being submitted for the Degree of Doctor of Philosophy in the School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, and has never been submitted before for any degree or examination in this University or any other university.



SHEHU, Asmau Muhammad

1st day of October 2025

Dedication

This thesis is dedicated to God Almighty; the most gracious the most merciful. To my late mother Hajiya Hadiza Hassan, my hero, May God Almighty grant you Jannah, Amin

My husband Dr Salihu and Children; Ahmad and Amal, thank you for your prayers, support, patience and encouragement.

Presentations arising from this study

1. Immunohistochemical Distribution of Glial Fibrillary Acidic Protein (GFAP) in three Developmental ages in the Brain of male Japanese Quail *(Coturnix Japonica*). International Society for Neurochemistry (ISN-APSN), 2022 Meeting, Honolulu HI, USA (Hawaii Convention Center), 28th August- 1st September 2022 (virtual presentation)
2. Achucarro International Glia School 2021: Understanding glial cell biology in health and disease. Achucarro Basque Centre for Neuroscience, Loao, Spain, 17-27 May 2021: Overview of gliogenesis in the Avian specie.
3. Age-related Distribution of S100 protein and glial fibrillary acidic protein (GFAP) in the male Japanese quail *(Coturnix Japonica*) Brain. The 15th International Meeting of the Society of Neuroscientists of Africa (SONA)/ 4th Ghana Neuroscience Society, Hybrid conference. Accra, Ghana. 27th – 30th October 2021 (Poster presentation).
4. Immunohistochemical localization of Neural Stem/Progenitor cells and Proliferation in the Telencephalon of the male Japanese quail *(Coturnix Japonica*). International Brain Research Organization (IBRO). IBRO Neural Stem School, held in Ibadan, Nigeria, 30th November- 4th December 2021 (Oral presentation).
5. Age-related Distribution of Oligodendrocytes in the male Japanese quail *(Coturnix Japonica).* The Society of Neuroscience (SFN), Virtual Neuroscience Meeting 2021, McCormick Place, Chicago, Illinois, USA. Poster No. 282.01/D16. 2018 (Virtual poster presentation)

Publications arising from this study

Manuscripts ready for submission

Abstract

Gliogenesis is a process that involves the generation of new glial cells (astrocytes, oligodendrocytes, and microglia) from neural stem cells (NSCs) or neural progenitor cells (NPCs) in both developing and adult individuals. The process of gliogenesis is essential for the preservation of the functional integrities of the central nervous system (CNS) from development to adulthood. The present thesis aimed to study gliogenesis and neurogenesis in the male Japanese quail (*Coturnix japonica*) brain at three different developmental stages: juvenile, subadult, and adult. This line of study used a multidisciplinary approach including histology (Nissl and myelin silver staining), revealed the cytoarchitecture in different brain regions, myelination fibers and patterns. Immunohistochemistry (IHC) using markers for neural and glial progenitors (SOX2), cell proliferation (PCNA), astrocytes (GFAP, S100A1), oligodendrocytes (OLIG2), microglia (IBA-1) and immature neurons (DCX). Immunofluorescence double-labeling IHC, which revealed colocalization of neural stem cells and GFAP the in ventricular zones, pial surface, optic tectum and cerebellum. Transmission electron microscopy (TEM) revealed detailed ultrastructural insights into mitochondria, glial and neuronal features. Findings highlight the distribution, characterization, timing, regional differences, and cellular mechanisms of gliogenesis, contributing to a deeper understanding of avian brain development. The study also showed dynamic neuronal and glial population changes during brain maturation and indicate a process of temporal and spatial regulation of gliogenesis during brain maturation.

These insights contribute to a greater understanding of cellular dynamics within the quail brain and, thus, are important for our more comprehensive understanding of avian development and brain evolution.

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List of abbreviations

Abbreviation	Meaning
A	Arcopallium
AC	Anterior Commissure
AIF-1	Allograft Inflammatory Factor 1
APC	Astrocytes Progenitor Cell
ATP	Adenosine Triphosphate
BAM	Border-Associated Macrophages
BBB	Blood-Brain Barrier
BDNF	Brain Derived Neurotrophic Factor
BSTL	Lateral Part of the Bed Nucleus of the Stria Terminalis
BSTM	Medial Part of the Bed Nucleus of the Stria Terminalis
CAM	CNS-Associated Macrophages
CAS	Central Animal Services
CB	Cerebellum
CBI	Nucleus Cerebella Internus
CBL	Nucleus Cerebella Lateralis
CDL	Dorsolateral Cortical Area
CNS	Central Nervous System
CP	Posterior Commissure
CPi	Piriform Cortex
DAB	Diaminobenzidine
DCX	Doublecortin
DL	Dorsolateral Region of the Hippocampus
DLA	Dorsolateral Anterior Thalami
DLL	Nucleus Dosrsolateralis Anterior Thalami, Pars Lateralis
DLM	Nucleus Dosrsolateralis Anterior Thalami, Pars Medialis
DM	Dorsomedial Region of the Hippocampus
DMA	Nucleus Dorsomedial Anterior Thalami,
E	Entopallium
EPL	External Plexiform Layer of the Olfactory Bulb
EW	Nucleus of Edinger-Westphal
FPL	Fasciculus Prosencephalic Lateralis
Gcl	Granular Cell Layer
GCt	Griseum Centrale
GFAP	Glial Fibrillary Acidic Protein
GL	Glomerular Layer
GLv	Nucleus Geniculatus Lateralis, Pars Ventralis
GM	Grey Matter
GP	Globus Pallidus
HA	Hyperpallium Apicale
HD	Hyerpallium Densocellulare
HM	Nucleus Habenularis Medialis
HL	Nucleus Habenularis Lateralis
HP	Hippocampus

HVC	High Vocal Centre
IBA-1	Ionized Calcium Binding Adopter Molecule 1 Antibody
IGrL	Internal Granular Layer of the Olfactory Bulb
IMc	Nucleus Isthmi, Pars Magnocellularis
INP	Nucleus Intrapeduncularis
IPc	Nucleus Isthmi, Pars Parvocellularis
LA	Nucleus Lateralis Anterior Thalami
LHy	Lateral Hypothalamus
Ll	Lateral Layer of the Ventral Hippocampus
LM	Nucleus Lentiformis
LSt	Lateral Striatum
LV	Lateral Ventricle
M	Mesopallium
MCL	Mitral Cell Layer of the Olfactory Bulb
Ml	Medial Layer of Ventral Hippocampus
MLd	Nucleus Mesensephalicus Lateralis, Pars Dorsalis
Mm	Mammillary Region
MSt	Medial Striatum
N	Nidopallium
N VII	Facial Nucleus
nBOR	Nucleus of the Optic Root
NC	Nidopallium Caudale
NDB	Nucleus Diagonalis Broca
NGF	Nerve Growth Factor
NGS	Normal Goat Serum
NIH	National Institute of Health
nIII	Oculomotor Nerve
NSCs	Neural Stem Cells
NSPC	Neural Stem/Progenitor Cells
OBV	Olfactory Bulb
OC	Optic Chiasm
OLIG2	Oligodendrocyte Lineage Transcription Factor 2 Antibody
OMN	Oculomotor Nucleus
ON	Olfactory Nerve
ONL	Olfactory Nerve Layer
OPCs	Oligodendrocyte Progenitor Cells
OV	Ovoid Nucleus
PB	Phosphate Buffer
Pcl	Purkinje Cell Layer
PCNA	Proliferating Nuclear Cell Antigen
PL	Nucleus Pontis Lateralis
PMH	Nucleus Medialis Hypothalamic Posterioris
PO	Preoptic Region
POM	Preoptic Nucleus
PPC	Nucleus Principalis Precommissuralis
PPN	Pedunculopontine Tegmental Nucleus
PVM	Nucleus Periventicularis Magnocellularis

RGCs	Radial Glial Cells
RMC	Red Nucleus Magnocellularis
RSv	Nucleus Reticularis Superior, Pars Ventralis
RT	Nucleus Rotundus
Ru	Nucleus Ruber
SAC	Stratum Album Centrale
SCi	Stratum Cellular Internum
SEZ	Subependymal Zone
SGFS	Stratum Griseum et Fibrosum Superficiale
SGC	Stratum Gracile Centralis
SL	Septal Nucleus Lateralis
SM	Septal Nucleus Medialis
SNc	Substantia Nigra, Pars Compacta
SNr	Substantia Nigra, Pas Reticulata
SO	Stratum Opticum
SOX2	SRY-related High Mobile Gene 2
SP	Nucleus Subpretectalis
SPC	Nucleus Superficialis Parvocellularis
SpL	Nucleus Spirformis Lateralis
SRt	Nucleus Subrotundus
STN	Subthalamic Nucleus
SVZ	Subventricular Zone
TEM	Transmission Electron Microscope
TeO	Optic Tectum
TPO	Temporo-Parieto-Occipital Area
Tr	Triangular Area of Ventral Hippocampus
TrO	Optic Tract
TU	Nucleus Tuberis
V	Ventricle
VMN	Ventromedial Nucleus
VP	Ventral Pallidum
VT	Vasotocinergic Cells
VZ	Ventricular Zones
WM	White Matter

CHAPTER ONE: Introduction

1.1 Background on gliogenesis

Gliogenesis refers to the generation of new glial cells, including astrocytes and oligodendrocytes, originating from neural stem cells (NSCs) or neural progenitor cells (NPCs) in both developing and adult organisms (Arai and Lo, 2017; Lanjewar and Sloan, 2021). This process is vital for maintaining the functional integrity of the central nervous system (CNS) from the developmental stages through adulthood (De Melo *et al.,* 2016). Gliogenesis is a multifaceted process that encompasses proliferation, differentiation, migration, and maturation of newly formed cells (De Melo *et al.,* 2016; Bekiari *et al.,* 2020; Lanjewar and Sloan, 2021). This occurs concurrently with or after neurogenesis. However, glial cells (astrocytes and oligodendrocytes) and neuronal cells arise from the same neural progenitor cells (NPCs) within the CNS (De Melo *et al.,* 2016). In contrast, microglia are derived from hematopoietic stem cells located outside the CNS and migrate to the CNS during embryogenesis (Cuadros *et al.,* 2011; Cuadros *et al.,* 2022).

The process of gliogenesis is increasingly recognized as significant in the field of neurobiology; however, its comprehensive mechanisms, including regulatory processes, remain insufficiently understood (Arai and Lo, 2017; Zhang *et al.,* 2020). The intricate interrelationship between neurons and glial cells is essential for the central nervous system (CNS) function. Glial cells play several roles in the CNS including myelination, immune activity, structural support, and metabolic homeostasis (Milichko and Dyachuk, 2020). Their involvement in cognitive processes, such as memory, is yet to be definitively established (Fields *et al.,* 2014; Henn *et al.,* 2022).

Dysfunction of glial cells has been associated with various developmental, neurological, and cognitive disorders in aging, as well as neuropathological conditions such as multiple sclerosis and schizophrenia (Henn *et al.,* 2022; Laricchiuta *et al.,* 2024). Gliogenesis is strictly regulated by both extrinsic and intrinsic signals to which cells are exposed, such as hormones, ischemia, brain disease, and stress (De Melo *et al.,* 2016). Some factors that regulate gliogenesis in adults are also involved in neurogenesis, revealing a close interrelationship between the two processes. Exercise exerts regulatory effects on hippocampal gliogenesis and neurogenesis, possibly by increasing levels of neurotrophins in the brain, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (De Melo *et al.,* 2016). Gliogenesis has been examined in the hippocampal formations of rodents (Niklison-Chirou *et al.,* 2020), catsharks (*Scyliorhinus canicular*) (Docampo-Seara *et al.,* 2019), and invertebrate species, including Drosophila (*Drosophila melanogaster*), grasshoppers (*Schistocerca gregaria*) (Liu, 2013), and worms (*Caenorhabditis elegans*) (Zhang and Yan, 2022).

1.2 Gliogenesis in avian species

In avian species, most studies on gliogenesis have focused on the localization and quantification of glial cells in the optic nerve and retina of quails and chickens (Fischer, Zelinka, & Scott, 2010; Wilken & Reh, 2016; Laricchiuta *et al.,* 2024), the auditory pathway of the chick brain stem (Dinh *et al.,* 2014), satellite glial cells in the telencephalon of Caledonian crows and Passeriformes birds (Medina *et al.,* 2013), microglia in the hippocampus of pigeons and migratory sandpiper species, astrocytes in the optic tectum of chickens (Lever *et al.,* 2014), oligodendrocytes in the avian retina (Yamagata, Yan, & Sanes, 2021), and the spinal cord segment in quails (Cakmak and Karadag, 2019).

Despite these investigations into gliogenesis across various species, there remains a deficiency in the systematic characterization of gliogenesis potential throughout the brains of adult avian species across different life stages. Furthermore, there is a notable absence of concurrent examinations of gliogenesis and adult neurogenesis throughout the brains of avian species.

1.3 Birds as a model for gliogenesis

Birds display complex behaviors, such as using tools, solving problems cooperatively, mimicking sounds, and singing, which are on par with or even exceed those of other mammals that do not have six-layered neocortices (Barnea and Pravosudov, 2012). In addition, birds engage in behaviors such as food storage, migration, and establishment of social hierarchies, which are not as commonly observed in mammals (Kashetsky *et al.,* 2021).

These characteristics make birds' ideal candidates for neuroscience studies, particularly when examining developmental, cognitive, and age-related neuropsychiatric disorders. The process of adult neurogenesis, which involves the creation of new neuronal cells in adult organisms, has been thoroughly studied in various bird species, including songbirds (Larson, 2020; Owji and Shoja, 2020; Aronowitz *et al.,* 2021; Rundstrom and Creanza, 2021) and non-songbirds (Mazengenya *et al.,* 2020). Moreover, numerous studies have shown that adult neurogenesis in vertebrates tends to decline with age (Kase *et al.,* 2020; Zocher and Toda 2023). Despite extensive research on adult neurogenesis in birds, there is limited information on the gliogenesis pathways in these species. Therefore, the aim of the current study was to explore and detail the different stages of potential gliogenesis and compare them to the processes occurring during neurogenesis in the brains of juvenile, subadult, and adult male Japanese quail (*Coturnix japonica*).

1.4 The relevance of studying birds to neuropsychiatric disorders

Studying birds holds significant potential for advancing our understanding of neuropsychiatric disorders due to their unique biological and physiological traits. Birds offer valuable insights in several areas that are relevant to neuropsychiatric research:

Behavioral and Social Complexity: Birds exhibit intricate social behaviors and complex communication systems similar to those found in mammal (Austad, 2011; Burish et al., 2004; Meisner et al., 2024; Nomura & Izawa, 2017; Willemet, 2013). These behaviors make them excellent models for studying the neural mechanisms underlying social interactions, communication, and cognitive processes, which are often disrupted in neuropsychiatric conditions (Adkins-Regan, 2009; Azarias et al., 2025; Gandhi & Lee, 2021; Henry et al., 2015; Konishi et al., 1989; Manduca et al., 2021; Nomura & Izawa, 2017; Sato et al., 2023).

Genomic Insights: The avian genome is characterized by a high degree of evolutionary stasis, providing a clear framework for comparative studies(Boudriot et al., 2025; Dauncey, 2013; Elvsåshagen et al., 2020; Griffin et al., 2024; Leonardsen et al., 2023; Parikshak et al., 2015; Smeland et al., 2023; Van Doren et al., 2017; Yang et al., 2021; You et al., 2024; Zhang et al., 2014). This stability, combined with specific evolutionary adaptations, allows researchers to explore genetic factors linked to brain functions and disorders. Understanding these genetic mechanisms can help identify potential therapeutic targets across species (Zhang et al., 2014).

Olfactory Research: Birds have sophisticated olfactory systems, which are pertinent given the emerging evidence of olfactory deficits as early indicators of neuropsychiatric disorders (Corfield et al., 2015; Debose & Nevitt, 2008; Grieves et al., 2022; Marin et al., 2023; Steiger et al., 2008; Yang et al., 2023).

This attribute can facilitate the study of sensory processing and its association with mental health conditions, aiding in early detection and intervention (Yang et al., 2023).

Functional Mitochondria in Erythrocytes: Unlike mammals, bird erythrocytes contain functional mitochondria (Bonfanti & Peretto, 2006; Li et al., 2023; Merkle & Alvarez-Buylla, 2006; Tan et al., 2023). This unique feature allows for the study of mitochondrial function and oxidative stress in relation to neuropsychiatric disorders (Delhaye et al., 2016; Shaki et al., 2017; Stier et al., 2013; Tsutsui et al., 2008; Woo et al., 2021; Yang. N et al., 2020; Yang et al., 2024). The ability to assess mitochondrial activity in avian blood samples offers a promising model for investigating the role of cellular metabolism in brain health (Stier et al., 2013).

Microbiota Studies: The gut-brain axis is a critical area of research in understanding neuropsychiatric disorders. Birds, with diverse and complex gut microbiota (Ahmed et al., 2024; Escobar et al., 2022; Generoso et al., 2020; Gruenbaum et al., 2024; Kim, 2023; Louwies et al., 2019; Mu et al., 2016; Ohara & Hsiao, 2025; Schneider et al., 2024; Sherwin et al., 2016; Socała et al., 2021), provide a model for exploring how these microbial communities might influence brain function and behavior, offering clues to potential microbiome-targeted therapies (Grond et al., 2018).

Disease Transmission Models: Birds often serve as natural reservoirs for diseases, including avian influenza, which can affect neural functions. (Causey & Edwards, 2008; Pantin-Jackwook & Swayne, 2009; Puryear & Runstadler, 2024; Verhagen et al., 2017; L. Zhang et al., 2023) Studying these interactions can shed light on the impact of infections and immune responses on neuropsychiatric health (Franca & Brown, 2014). Together, these attributes make birds valuable models for neuropsychiatric research, offering unique angles to unravel complex brain functions and their disorders.

1.5 Relevance of the Japanese quail to neuroscience research

The Japanese quail species was developed through domestication of the common quail in China and arrived in Japan in the 11th or 12th century. Originally bred as domestic songbirds, the Japanese quail became popular in the 20th century for meat and egg production (Ainsworth et al., 2010). Japanese quail were first reported as a useful research model by Padgett and Ivey (1959) and since then have become a common laboratory species.

Japanese quail are emerging as an animal model for multiple areas of scientific inquiry such as study of birth defects and diseases that affect human health, human hereditary diseases, malformations, and abnormalities (Cheng and Kimura, 1990, Mizutani, 2002, Tsudzuki, 2008). Quail have been used in studies addressing senescence in immunology, endocrinology, developmental (Dickman et al., 2004) and reproductive biology (Ottinger et al., 2004) hypercholesterolemia characterized by the development of vascular lesions and xanthomatosis (Murakami et al., 2010) glycogen storage disease (Matsui et al., 1983), myotonic dystrophy and acid maltase deficiency, known as Pompe's disease (Mizutani, 2002).

The Japanese quail remains a powerful research model for many biological processes including, reproductive events and aging (Padgett and Ivey, 1959, Follett et al., 1992, Huss et al., 2008), brain development (Marasco et al., 2016), neuroanatomical and neuronal functions studies (Castagna et al., 2003), variety of human genetic disorders (Baer et al., 2015) and as potent ontogenetic development models (Mills et al., 1997) to mention but a few.

The advantage of using quail as research subjects and laboratory animals is because of the following factors: because of their precocious nature, they require minimal parental care; their small size requires minimal maintenance in terms of housing and feeding; their short lifespan enables physiologic and aging studies to be conducted within a short period of time; they have a very high egg production, averaging 250 eggs per annum. They are capable of producing 3-4 generations per annum; quail exhibit specific genetic and phenotype characteristics leading to specific quail lines, which reduce confounding factors of genetic variability and a low-cost alternative to other commonly used laboratory species such as rats and mice (Huss et al., 2008, Jaspers, 2015).

Japanese quail are a viable alternative to rodents for the study of sex differences and drug abuse (Alvino et al., 2009, Mills et al., 1997). More additional advantage of using the Japanese quail for adult gliogenesis research is because it can be bred easily in the laboratory, low cost compared to rodents and other species, and accessibility of transgenic lines. Genetically unique strains of Japanese quail have been used to study the visual system; developmental pathology of the retina and optic nerve, spontaneous glaucoma cataracts, and retinal degeneration (Zak et al., 2010) are some of the topics that have been investigated and they also exhibit spatial orientation and memory to recall specific sites (Ruplo et al., 2011). Over the last 50 years, the Japanese quail has proven to be a truly diverse and efficient animal model.

Thus, domesticated Japanese quails offer a viable alternative to rodents and mice as research model animals in biomedical research. In the future, it is likely that the Japanese quail will continue to occupy a small yet prominent place in research laboratories around the world.

Rats, mice and songbirds have been extensively studied in adult neurogenesis, however, there are few or paucity of literature on adult gliogenesis in the avian species therefore making the Japanese quail the ideal choice for my PhD thesis.

1.6 Gender variation in study animals and its influence on neuroscience studies

There is a complex interaction between hormones and neurogenesis, both in mammals and in birds (Galea 2008). The study of brain/behaviour relationships in birds are much easier than in mammals in which hormone target tissues like the limbic system are involved in regulating many different physiological processes and behaviours (Barnea and Pravosudov, 2011).

In birds sex steroids hormones do not appear to regulate the rate of cell division in the Ventricular Zone (Brown et al., 1993). However, testosterone has a pronounced and rapid effect on higher vocal centre (HVC) and Area X, respond more slowly. Prolactin in female bird and mammals mediates an increase in neurogenesis in the subventricular zone (SVZ) (Shingo et al., 2003, Larsen et al., 2008). Oestrogens also influence neuronal differentiation and survival in the avian brain, but do not seem to directly influence cell proliferation (Lee et al., 2007). Williams et al. (1999) demonstrated that oestrogens also promote the initial migration of new neurons from SVZ explants *in vitro*.

The convergence of evidence suggests that oestrogens act as neurotrophic agents and supply migratory support for the newly-formed neurons in reaching their end destinations (Lee et al., 2007). Ball and Balthazart (2010) also reported that male and female quail differ markedly in the way their brain responds to sex steroid hormones. Therefore, adult neurogenesis and possibly gliogenesis is affected by sex steroids hormones, so it is prudent to examine the process in one sex to minimize the effects of sex hormones.

1.7 Aim of the thesis

This research examined the process of gliogenesis in the male Japanese quail brain, with the aim of establishing a comprehensive understanding of how neural stem/progenitor cell activity, proliferation rates, and differentiation patterns evolve with age. This study investigated age-related changes in gliogenesis in male Japanese quail brains, focusing on the localization of neural stem/progenitor cells, cell proliferation, and differentiation patterns across developmental and postnatal stages.

1.8 Specific objectives of the thesis

1. To provide a detailed microscopic analysis of the regional subdivisions in the juvenile, subadult and adult male Japanese quail brain in serial sectioned material using Nissl stain and Myelin silver stain.
2. To investigate in detail the potential of gliogenesis in the juvenile, subadult and adult male Japanese quail brain using single labelling immunohistochemistry:
    a. To assess the distribution of neural stem/progenitor cells (NSPC) in different brain regions using SRY-related high mobile gene 2 (SOX2) antibody.
    b. To determine the presence of Proliferating cell nuclear antigen (PCNA) antibody.
3. To define the lineage pattern of identifiable glia and neuronal cells expressed by the and the following differentiation marker were used:
    a. For Astrogliogenesis: Glial fibrillary acidic protein (GFAP) and calcium-binding protein (S100A1) antibody.
    b. For Oligodendrogenesis: Oligodendrocyte lineage transcription factor 2 (OLIG2) antibody.

c. For Microgliogenesis: Ionized calcium binding adopter molecule 1 (IBA-1) antibody.

4. To investigate the potential of gliogenesis and neurogenesis in the juvenile, subadult and adult male Japanese quail brain using double labelling immunohistochemistry:
    a. To determine the distribution of glia and neuronal cells in the quail brain through immunofluorescence.
    b. To evaluate the possible colocalization of gliogenic and neurogenic cells.
    c. To describe the potential colocalization of neural stem cells with gliogenic and neurogenic cells.
5. To describe ultrastructural elements identified by the transmission electron microscope (TEM) as follows:
    a. To identify ultrastructure of glia and neurons in the juvenile, subadult and adult brain regions.
    b. To elucidate the morphology and distribution of mitochondria in the brain.

In conclusion, my research aimed to comprehensively investigate gliogenesis in the brains of male Japanese quails at different developmental stages. By examining neural stem and progenitor cells, cell proliferation, migration and differentiation in juvenile, subadult and adult quails. The research provided a valuable insight into age-related changes in gliogenesis. The multifaceted approach, which combines detailed microscopic analysis, immunohistochemistry, confocal fluorescence microscopy, and transmission electron microscopy, offers a thorough characterization of glial and neuronal cells throughout the quail brain. My thesis has addressed a significant gap in our understanding of avian gliogenesis and its relationship with neurogenesis.

The findings have also enhanced our knowledge of glia throughout postnatal development, health, and aging in important avian model species.

CHAPTER TWO: Literature Review

2.1 Adult gliogenesis in the avian brain

Gliogenesis refers to the generation of new glial cells, specifically astrocytes and oligodendrocytes, from neural stem cells or neural progenitor cells (NPCs) in both developing and adult organisms (Arai and Lo, 2017). This process is essential for maintaining the functional integrity of the central nervous system from developmental stages through the adult life of an organism (Nakafuku, 2020). Gliogenesis is a multistep process characterized by the proliferation, differentiation, migration, and maturation of newly formed cells (Arai and Lo, 2017). It occurs during or after the termination of neurogenesis (Arai and Lo, 2017; Fu *et al.,* 2021). The capacity of the adult brain to adjust to specific environmental changes depends on the interplay between adult neurogenesis and gliogenesis (Arai and Lo, 2017). While gliogenesis is gaining traction in the field of neurobiology, its full process, including the mechanisms that regulate it, remains unclear (Fischer *et al.,* 2010). The intricate relationship between neurons and glial cells is crucial for the functioning of the central nervous system (CNS) (Bittern *et al.,* 2021).

Gliogenesis is tightly controlled by both external and internal signals that cells encounter, such as hormones, ischemia, brain disorders, and stress (Niklison-Chirou *et al.,* 2020; Zhang, Noma, and Yan, 2020). Certain factors that influence gliogenesis in adults also play a role in adult neurogenesis, highlighting a strong connection between these two processes (Biswas *et al.,* 2020).

Physical activity can regulate both hippocampal gliogenesis and neurogenesis, potentially by elevating levels of neurotrophins in the brain, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (De Melo *et al.,* 2016).

Glial cells in the central nervous system perform various roles, including myelination, immune response, structural support, and maintaining metabolic balance (Vasile *et al.,* 2017; Borst *et al.,* 2021). Nevertheless, their involvement in cognitive functions, such as memory, remains uncertain (Augusto-Oliveira *et al.,* 2020). Malfunctioning glial cells have been associated with numerous developmental, neurological, and cognitive disorders, particularly prevalent in aging and neuropathological conditions like multiple sclerosis and schizophrenia (Notter, 2021; Papiri *et al.,* 2023).

Birds exhibit intricate behavioral repertoires, including tool use, cooperative problem-solving, vocal mimicry, and bird song, which are comparable to or surpass those of other mammals (Kashetsky *et al.,* 2021), despite lacking six-layered neocortices. Additional behaviors observed in birds, in contrast to mammals, encompass food hoarding, migration, and social dominance (Wood *et al.,* 2022). These behaviors render bird exemplary candidates for neuroscience research as animal models for developmental, cognitive, and age-related neuropsychiatric conditions.

Considering this premise, adult neurogenesis, defined as the generation of neuronal cell lineages in adult species, has been extensively investigated in various avian species, including songbirds (Larson, 2020; Owji and Shoja, 2020; Aronowitz *et al.,* 2021; Rundstrom and Creanza, 2021) and non-songbirds (Mazengenya, Bhagwandin, and Ihunwo, 2020). Numerous studies have demonstrated that adult neurogenesis declines with age across vertebrate species (Kase *et al.,* 2020; Zocher and Toda, 2023).

Understanding the age-dependent variations in adult neurogenesis has been instrumental in further elucidating the role of glial cells in neurological diseases (Culig, Chu, and Bohr, 2022).

The investigation of gliogenesis in avian species has concentrated on the localization and quantification of glial cells within various neural structures. Specifically, research has examined the optic nerve and retina in quails and chickens (Lever, Brand-Saberi, and Theiss, 2014; Xiao *et al.,* 2020), the auditory pathway in the chick brainstem (Cramer and Rubel, 2016), and satellite glial cells in the telencephalon of Caledonian crows and Passeriformes (Medina *et al.,* 2013; Giglia *et al.,* 2022). Additionally, studies have focused on microglia in the hippocampus of pigeons and migratory sandpiper species, astrocytes in the optic tectum of chickens, oligodendrocytes in the avian retina (Yamagata, Yan, and Sanes, 2021), and the spinal cord segment in quails (Cakmak and Karadag, 2019).

2.2 Radial glial cells (RGCs) as neural stem cells in adult gliogenesis

Radial glial cells (RGCs) were initially identified by Golgi (1885) and Magini (1888) as bipolar cells characterized by radial extensions, a subpial end foot, and a cell body located just beneath the ventricle. These cells serve as structural support for the migration of newly formed neurons to the upper layers of the cortex (Bentivoglio and Mazzarello, 1999; Arellano *et al.,* 2021). In addition to producing various types of glial cells, RGCs function as neuronal progenitor cells, giving rise to neurons during the central nervous system's development (Arellano *et al.,* 2021). These cells remain present into adulthood, confined to specific brain regions such as the subependymal zone (SEZ) along the lateral wall of the lateral ventricle, the walls of the third ventricle in the hypothalamus, and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus (Bentivoglio and Mazzarello, 1999; Lange *et al.,* 2020).

Radial glial cells (RGCs) have been consistently found in brain areas beyond the subependymal zone (SEZ) and subgranular zone (SGZ) in both invertebrates, such as in mushroom bodies, and vertebrates, including the neocortex, striatum, cerebellum,

amygdala, and substantia nigra. Studies across various species on the evolutionary spectrum, including crayfish and teleosts (Chaves Da Silva *et al.,* 2013), lampreys, adult zebrafish (Lange *et al.,* 2020), amphibians (D'Amico et al., 2011; Miranda-Negrón and García-Arrarás, 2022), reptiles (Moreno and González, 2017), and birds, have been conducted. In adult species, during gliogenesis, RGCs multiply to form either oligodendrocyte or astrocyte lineages. The oligodendrocyte lineage involves RGCs undergoing cell proliferation, migration, and differentiation into pre-oligodendrocytes and immature, mature unmyelinated, and mature myelinated oligodendrocytes.

Conversely, the astrocytic lineage generates astrocyte progenitors that mature into immature and mature astrocytes (Beiter *et al.,* 2018; Beiter *et al.,* 2022). Although oligodendrogenesis and astrogenesis appear to occur independently and produce distinct cell lines, it remains unclear whether the radial glial cells contributing to these lineages are different. Similarly, the radial glial cells that participate in the neural lineage to produce new neurons in adults resemble glial progenitors (Miranda-Negrón and García-Arrarás, 2022). Therefore, examining the events of postnatal gliogenesis and neurogenesis together could provide deeper insights into these processes.

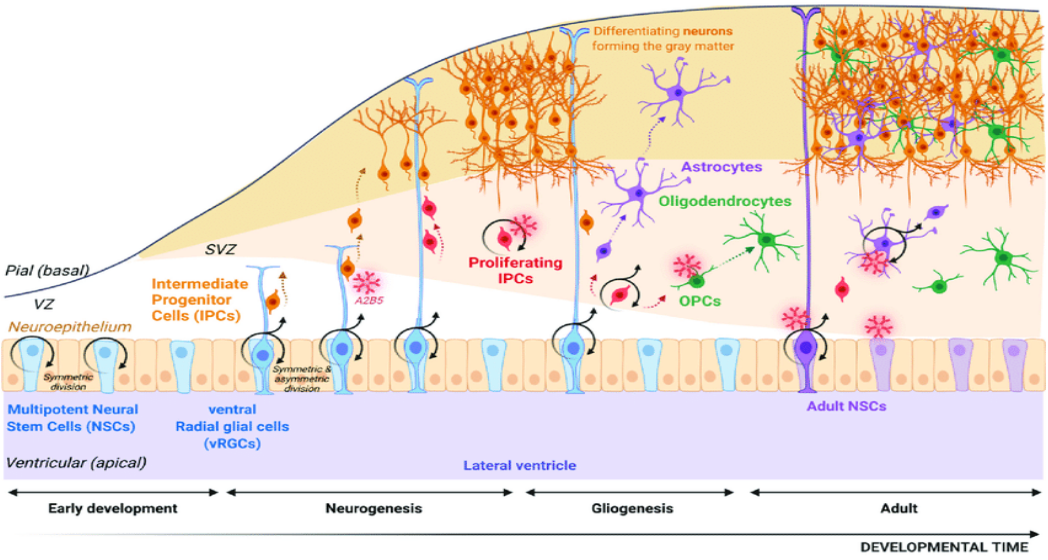


Figure 2.1: Neuro-Gliogenesis via Lateral Inhibition

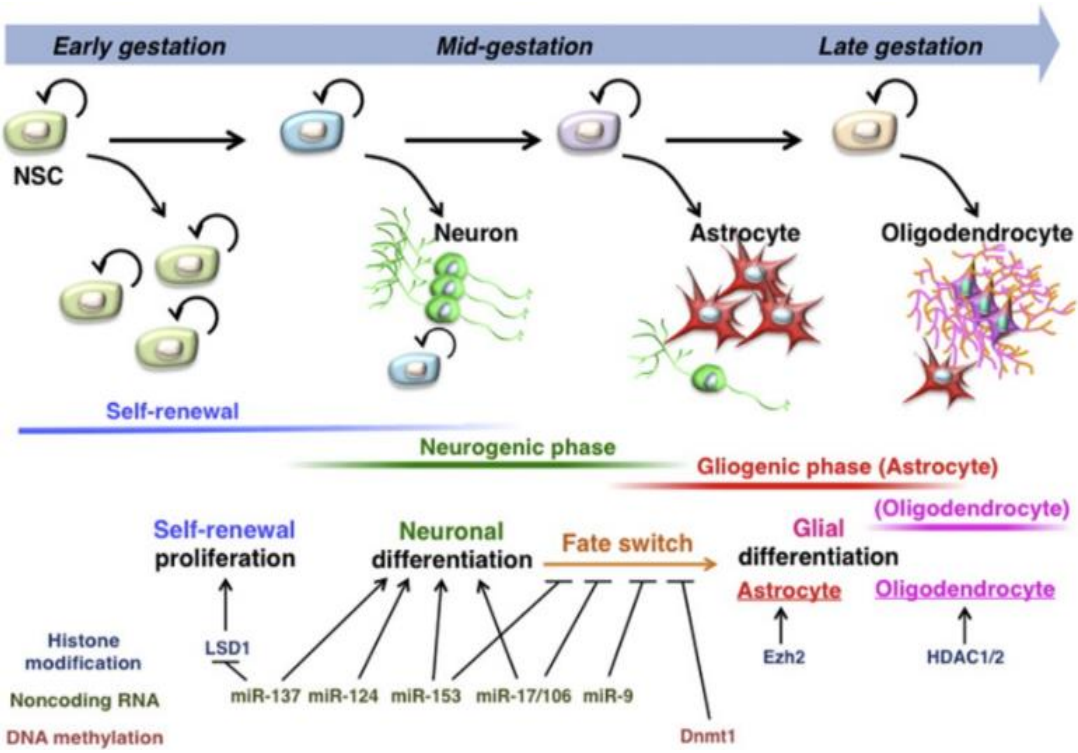


Figure 2.2: Neurogenesis and Gliogenesis Processes During Development

In neurogenic niches, RGCs that express Glial fibrillary Acid Protein (GFAP) play a role in boosting proliferation and enhancing vascular communication (Ma *et al.,* 2005; Ridaura *et al.,* 2021). Radial astrocytes are divided into two categories based on their structural characteristics. Type A features a long, sparse process that proliferates to form neurons, whereas Type B, which is less prevalent, consists of less branched radial astrocytes that are crucial components of neurogenic niches across all adult vertebrates (Schneider *et al.,* 2019; Yeh *et al.,* 2023). In adult birds and mammals, the cell bodies of actively dividing radial astrocytes are in the SEZ and SVZ of the telencephalon (Alvarez-Buylla and Nottebohm, 1988; Dimou and Götz, 2014). Studies on adult birds such as zebra finches, quails, chickens, and canaries have shown that radial astrocyte cell bodies are positioned in the ventricular zone of the lateral forebrain ventricles, with radial fibers extending into the forebrain parenchyma (Alvarez-Buylla and Nottebohm, 1988).

2.3 Role of mitochondria and mitochondrial metabolism in neural stem cell (NSC)

Neural stem cell proliferation in adult hippocampal neurogenesis and gliogenesis provides the first evidence that cell lineage continuation and its functional roles are related to metabolic programs (Knobloch *et al.,* 2013). Both developmental and adult gliogenesis and neurogenesis expend energy during proliferation, differentiation, and maturation (Hsieh and Zhao, 2016). The energy utilized in these stages originates from the mitochondria during metabolism. Metabolic energy is also associated with apoptosis and calcium homeostasis (Bond *et al.,* 2015; Beckervordersandforth *et al.,* 2017).

Extensive changes occur in neural stem cells during the transitional period from proliferative activities through differentiation (cellular growth and synaptic activity) into mature neurons (Shin *et al.,* 2015).

Neuronal maturation is dependent on energy (ATP) from the process of aerobic glycolysis for the continuation and regeneration of lineage potency (Lanza *et al.,* 2011; Bond *et al.,* 2015), and oxidative phosphorylation for the support and growing energy demands of a progeny (Zhang *et al.,* 2016; Khacho and Slack, 2018). Alterations in mitochondrial distribution and morphology affect neural precursor cell proliferation (Wakabayashi *et al.,* 2014) and neuronal survival (Ishihara *et al.,* 2009) during embryonic development. In the adult brain, a decrease in mitochondrial ATP production impairs the development of adult-born neurons, contributing to neuropsychiatric diseases and cognitive symptoms during aging (Beckervordersandforth *et al.,* 2017).

However, the recognition of metabolic activities at specific phases and their influence on adult neurogenesis, particularly in gliogenesis, are mostly unknown (Beckervordersandforth *et al.,* 2017). Mitochondrial metabolism is crucial for cell genesis, influencing cellular development, differentiation, and function. Recent studies highlight its role in shaping cellular outcomes. It is essential for energy production and biosynthesis, regulating cell fate (Hamanaka & Chandel, 2013). In stem cells, mitochondrial physiology links to pluripotency and differentiation. P19 stem cells show a glycolytic profile and reduced mitochondrial biogenesis compared to differentiated cells (Vega-Naredo *et al.,* 2014). This state is associated with resistance to compounds and pluripotency maintenance. Stimulating mitochondrial function in stem cells can induce loss of pluripotency and affect differentiation (Vega-Naredo *et al.,* 2014).

Mitochondrial dynamics, including fission, fusion, transport, and mitophagy, are vital for cell division, differentiation, and death (Xie *et al.,* 2020).

The mitochondrial network and cristae architecture impact cell differentiation and identity, especially during metabolic reprogramming in immune, stem, and cancer cells (Kawano *et al.,* 2023). Manipulating mitochondrial dynamics and cristae shape can affect T cell phenotype and macrophage polarization by altering energy metabolism (Kawano *et al.,* 2023). In conclusion, mitochondrial metabolism determines cell genesis, influencing stem cell pluripotency, differentiation, and identity. The plasticity of mitochondrial architecture and metabolism is crucial for reprogramming during cell development. Understanding these mechanisms could improve strategies for manipulating cell viability, differentiation, and identity (Kawano *et al.,* 2023).

2.4 Astrocytes

Astrocytes are the most prevalent cell type in the brain both during its development and in the mature central nervous system (CNS) (Freeman and Rowitch, 2013; Batiuk *et al.,* 2020). They exist in two stages: the proliferative stage, known as astrocyte progenitor cells (APC), and the adult non-proliferative mature stage (Sloan and Barres, 2014; Zhang *et al.,* 2016). These cells originate from radial glial cells, undergo proliferation, and produce new astrocytes (Reis *et al.,* 2007; Ge *et al.,* 2012a; Falk and Götz 2017).

2.4.1 Functions and morphology of astrocytes

Astrocytes play a crucial role in synapse formation, maintenance, and pruning. They control synapse genesis, development, and preservation, support brain metabolism, regulate calcium homeostasis, energy metabolism, and neurotransmitter recycling and release.

They also support myelin structures in white matter and maintain the blood-brain barrier (Emsley and Macklis, 2006; Ge *et al.,* 2012b; Clarke and Barres, 2013; Sloan and Barres, 2014; Allen and Eroglu, 2017; Verkhratsky and Butt, 2018; Tognatta *et al.,* 2020).

Notably, astrocytes are involved with adult neural stem cells and contribute to neurogenesis (Falk and Götz, 2017). Stensaas and Stensaas (1968) described and compared glial cell types in birds and turtles, identifying four types: ependymal cells, astrocytes (protoplasmic, fibrous, and Bergman), oligodendrocytes and microglia. These cells vary in size, quantity, location, and orientation, resembling those in mammals (Cajal, 1913; Garcia-Lopez *et al.,* 2010; Oberheim *et al.,* 2012).

In humans and primates, the primary glial subtypes are protoplasmic and fibrous cells. Two other specific types are interlaminar astrocytes in layer 1 of the murine cerebral cortex, comparable to those in other rodents (Colombo & Reisin 2004, (Marín-Padilla, 2015). Varicose projection astrocytes are exclusively found in layers 5 and 6 of the cerebral cortex and terminate on the vasculature (Sosunov et al., 2014). Miller and Raff (1984) identified two astrocyte subtypes in rodents based on location, morphology, and function. Protoplasmic astrocytes are found in grey matter with branched processes forming the glial limiting membrane, covering blood vessels and involved in glutamate clearance, synapse development, and elimination (Rothstein *et al.,* 1996; Oliet *et al.,* 2001; Kucukdereli *et al.,* 2011).

Fibrous astrocytes are in white matter with long unbranched processes, associated with blood vessels but with less understood functions (Marín-Padilla, 2011).

Research on other mammals has revealed different astrocyte classes, including Bergman, marginal, valate, perivascular, radial glial, protoplasmic, and fibrous types (Emsley and Macklis, 2006; Nag, 2011; Oberheim *et al.,* 2012; Olude *et al.,* 2015).

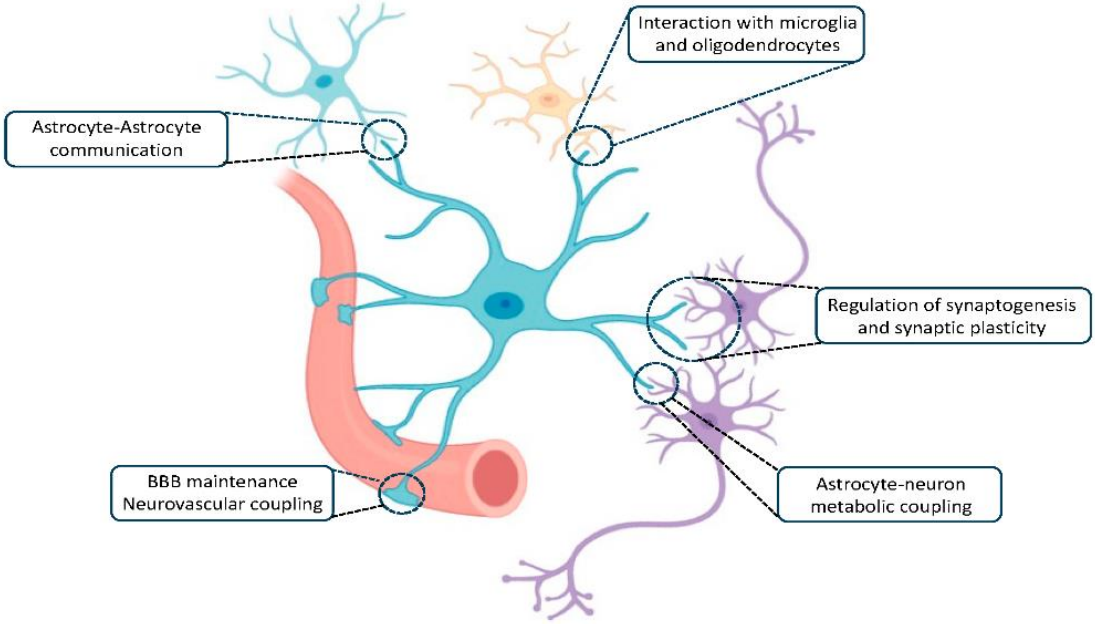


Figure 2.3: Functions and morphology of the astrocytes

2.4.2 Astrogliogenesis in the avian brain

Many studies focus on glial cell development in embryonic and early postnatal stages but understanding their roles in the adult brain is also crucial. Recent research explores mature glial functions in mammalian and avian brains. Linser and Perkins (1987) used immunohistochemical and biochemical techniques on chick optic tectum development, finding fibrous astrocytes with GFAP by day 9, and oligodendrocytes emerging later. Their work highlighted neuroglial interactions in the neural retinal system. Similarly, Seo *et al.* (2008) studied astrocyte formation and migration in the chicken optic tectum, noting tectal astrocytes migrate along retinal ganglion or radially, with vimentin proliferation decreasing and GFAP increasing with age.

Lever *et al.* (2014) examined glial and neuronal development in the chicken optic tectum, observing DCX, Tuj-1, and proliferation end at E3, E6, and E12. They noted neurons developed before hatching, with vimentin marking immature astrocytes at E4 and E6. Radial glial cells (RGC) appeared and transformed into astrocytes between E12 and E20. Vimentin-positive RGCs were bipotent, expressing in DCX cells between E4 and E6, with numerous synaptic densities in layer 5. These studies show astrocytes have diverse origins and migration paths and are heterogeneous. The avian optic tectum, homologous to the mammalian superior colliculus, is a well-established model for studying neurogliogenesis due to its manipulability (LaVail and Mullen, 1977; Lever *et al*., 2014). Gliogenesis is thought to occur after neurogenesis ends, but questions remain about how glial and neuronal cell lines diverge in the germinal zone, whether they occur simultaneously, or have different progenitors (Fujita and function, 2003; Fischer *et al.,* 2010; Norkutė *et al.,* 2010).

An immunohistochemical study aimed to determine the relative amount of astroglial cell lineage during early neurogenesis in the developing chicken brain. Findings showed that GFAP and GLAST were expressed as early as embryonic day E4, decreased by E7, and GFAP expression increased again after E7. These results suggest that gliogenesis occurs much earlier in the embryonic chicken brain than previously thought (Norkutė *et al.,* 2010). This is the first report showing GFAP is both expressed and translated during very early chicken brain development (Norkutė *et al.,* 2010). Similarly, research by Kommata and Dermon (2018) demonstrated vimentin is crucial for foliation and corticogenesis in the developing chick brain, localizing to radial and Bergmann glia. The expression pattern was like that in mammals (Buffo and Rossi, 2013).

These findings highlight the conservation of glial cell functions across vertebrate species, suggesting a common evolutionary origin. Comparative studies across avian species further expand our understanding of glial cell diversity and adaptations. Investigating the distribution of astrocytes in different bird species may reveal how these cells have evolved to support diverse cognitive and behavioral demands in various ecological niches.

2.4.3 Comparative studies across avian species

Comparative studies across various bird species, from chickens and quails to migratory sandpipers, have revealed both similarities and differences in glial cell distributions and functions. These interspecies comparisons provide valuable insights into the evolution and adaptation of glial cells in different ecological niches. Research on the distribution of astrocytes in avian brains predominantly focuses on developmental aspects.

Glial fibrillary acid protein (GFAP) serves as an astroglial marker indicating the cell population in birds. Cameron-Curry *et al.* (1991) conducted an in-depth investigation into GFAP distribution in the adult Japanese quail brain, employing both single and double labeling immunohistochemistry techniques. Their findings identified three astrocyte types based on their location and morphology in Japanese quails. Type 1 consists of unipolar, round cell bodies adjacent to large blood vessels. Type 2 cells are multipolar, varying in size with irregular cell bodies. Type 3 astrocytes are present in both gray and white matter. Additionally, Castagna *et al.* (2003) explored the distribution of various transcription factors, including S100, S100 alpha, and S100 beta, in both glial and neuronal cells in Japanese quails.

All antibodies exhibited strong glial-positive cells in the brainstem compared to the pallium. S100 and S100 alpha localized a few neurons in the pallium and subpallium. Few oligodendrocytes and radial glia were expressed, with no positive microglial localization. The S100 beta distribution pattern resembles that observed in rats (Rikmann and Wolf 1995). Glial cells in birds play a vital role in forming long-term memory (Gibbs *et al.,* 2011).

Migratory birds rely on enduring hippocampal memory to remember landmarks and migratory paths for their annual return to breeding grounds (Diniz *et al.,* 2016; Mouritsen *et al.,* 2016; Mettke-Hofmann, 2017; Carvalho-Paulo *et al.,* 2018; Lima *et al.,* 2019). Some studies on avian migration suggest a connection between neuronal recruitment in the hippocampus and migratory distance (Barkan *et al.,* 2014; Barkan *et al.,* 2016).

Most research on the hippocampal plastic response in memory formation has concentrated on neuron numbers and hippocampal volume (Diniz *et al.,* 2016; Mouritsen *et al.,* 2016; Mettke-Hofmann, 2017) concerning age, seasonal changes, food scarcity, and enriched environments (Mesquite 2016; Smulders 2017; Robertson 2017). However, there is limited information on the role of glial cells in hippocampal formation (Roth *et al.,* 2013; Herold *et al.,* 2018) and in migrating birds (Diniz *et al.,* 2016; Carvalho-Paulo *et al.,* 2018; Lima *et al.,* 2019). Seasonal migratory and wintering birds, such as the Sandpiper *Calidris pusilla*, possess three types of astrocytes differing in morphology and function (Carvalho-Paulo *et al.,* 2018).

Radial astrocytes contribute to neuronal development and migration (Kempermann *et al.,* 2015; Carvalho Paulo, *et al.,* 2018). Vascular astrocytes are associated with the blood-brain barrier (BBB), while stellate astrocytes are linked to the astrocytic network (Matyash and Kettenmann, 2010; McConnell *et al.,* 2017).

Carvalho-Paulo *et al.* (2018) conducted a study examining the potential role of glial cells and astrocytes in the hippocampal formation of migrating and wintering semipalmated sandpipers (*C. Pusilla*). They compared the number of cells in *C. Pusilla* before migration at the Bay of Fundy in Canada (249 cells) and after their extensive non-stop transatlantic flights (250 cells). The research employed optical fractionator, 3-D reconstruction (hierarchical cluster analysis), and GFAP immunostaining methods. Findings indicated the involvement of two astrocyte types: type I and type II. Morphological complexities in type I astrocytes of both migrating and wintering *C. Pusilla* were significantly less than those in type II astrocytes. In migrating birds, type I and type II astrocytes were 2.4 and 1.4 times more complex, respectively, than in wintering birds. The total astrocyte counts in long-distance wintering birds decreased

significantly from 76.7 % before migration to 63.8 % after wintering, while there was an increase from 23.3 % before flight to 36.4% after flight.

The study showed that type II astrocytes were less affected (72.5 %), closely associated with blood vessels, and potentially involved with the BBB, compared to the more affected type I astrocytes (25.5 %). The authors suggested that type I astrocytes might participate in different physiological functions due to their distance from blood vessels.

In a recent study by Lima *et al.* (2019), two migratory bird species, the semipalmated sandpiper (*C. Pusilla*), which undertakes a five-day non-stop transatlantic flight, and the semipalmated plover (*Ch. semipalmatus*), which flies overland with rest and feeding stopovers, were investigated to explore the relationship between astrogliogenesis (radial astrocytes) and neurogenesis under environmental influences. Hierarchical cluster, morphometric, and discriminant analyses were utilized. The study revealed significant morphological changes in type I radial astrocytes compared to type II radial astrocytes in both bird species. DCX-positive neurons were compared with RGL morphometrics in the V region of the hippocampal formation, showing a correlation between an increase in DCX-positive cells and the convex hull surface area of radial astrocytes. Additionally, there was a greater increase in RGL astrocytes in *Ch. Semipalmatus* than in *C. Pusilla*, possibly due to visuospatial environmental changes during overland flights. Thus, the findings indicated that flight mode influences radial astrocytes and neurogenesis (Lima *et al.,* 2019). A comparable investigation by Henrique et al. (2020) analyzed hippocampal astrocytes in *Ch. Semipalmatus* and *C. Pusilla* before and after autumn migration. The study found migratory flight affects Type I and II astrocytes differently.

In *C. Pusilla*, astrocyte numbers decreased significantly, while *Ch. Semipalmatus* showed no impact. Both species experienced morphological changes.

Phylogenetic difference did not affect results, as shown by the phylogenetic independent contrast method using nuclear and mitochondrial markers.

A study by da Costa *et al.* (2020) on *Arenaria interpres*, which undertakes a six-day long-distance non-stop migration, explored changes in hippocampal astrocytes during wintering and pre-migration. Findings indicated increased morphological complexity of type I and II astrocytes. Research has shown environmental factors influence astrocyte morphology during migration and wintering periods (Mendes *et al.,* 2013; Diniz *et al.,* 2016b; Carvalho-Paulo *et al.,* 2018). During long-distance flights, lipid reserves become crucial as brain glucose supply diminishes, increasing ketone body metabolism (Landys *et al.,* 2005).

Astrocytes play a key role in metabolism by absorbing, synthesizing, and releasing fatty acids as brain energy sources (Landys *et al.,* 2005; Achanta *et al.,* 2017). Glia-neuron interaction is essential for lipid metabolism (Barber and Raben, 2019). Studies on adult brain astrocytes have typically focused on specific regions like the hippocampus, ventricular zone, and optic tectum. However, examining the entire brain is necessary to understand processes in other areas and species.

2.4.4 Advanced research techniques to study astrocyte heterogeneity

The diversification of astrocytes is not fully understood, but modern molecular tools have advanced research into astrocyte heterogeneity. Astrocytes show unique molecular and functional characteristics, heterogeneity, morphology, proliferation, and migration, varying by maturation stage, microenvironment, and brain region (Köhler *et al.,* 2019; Matias *et al.,* 2019; Miller *et al.,* 2019; Shoneye *et al.,* 2020).

Shoneye *et al.* (2020) explored if temporal proliferation in the subcortex (hypothalamus) mirrors that in the cortex during postnatal stages using genetic and Edu labeling in transgenic mice. In the first postnatal week, the hypothalamus had few proliferating astrocytes. By P15–P30, these cells became more diverse, increased in percentage, and were stronger synaptogenic inhibitors than those in the cortex. Clonal analysis showed region-specific transcriptional and epigenetic signatures in neurons and astrocytes in the neocortex and thalamus. As growth occurs, progenitor cells are marked by gene expression programs conferring post-mitotic diversity (Herrero-Navarro *et al.,* 2020). Batiuk *et al.* (2020) identified five distinct astrocyte subtypes in the mouse cortex and hippocampus using single-cell RNA sequencing, aligning with Lozzi *et al.* (2020).

Research on astrocyte heterogeneity in morphology during aging in the olfactory bulb in mice used Sholl, 3D analysis, and GFAP staining in mice aged 3 months to 2 years. Three types were identified: type I (stellate), type II (elliptic), and type III (squid-like). Significant changes were observed from three months to one year; changes between 1-2 years were not significant. This study indicates astrocyte heterogeneity undergoes a morphological transition until late adolescence (Klein *et al.,* 2020).

Recently, methods like RNA-seq with ribosome pull-down (Chai *et al.,* 2017; Morel *et al.,* 2017), cell surface antigen-based sorting (Lin et al., 2017), and single-cell isolation techniques (Zeisel *et al.,* 2018) have highlighted the molecular diversity of astrocytes in the adult brain. Advanced techniques, including high-resolution imaging and optogenetics (Butt *et al.,* 2018), have been used to study their morphology during postnatal development (Felix *et al.,* 2020).

Similarities and differences between neonatal and postnatal astrocytes have been observed in receptors, signaling pathways, density, cellular structures, and extracellular spaces (Kim *et al.,* 2011; Schreiner *et al.,* 2014; Felix *et al.,* 2020). Investigating astrocyte heterogeneity in migratory and non-migratory avian species regarding their transcriptome programs in healthy and aged brains is necessary. This will provide insights into astrocyte diversification across species, while astrocytes are crucial for neuronal support and brain homeostasis, oligodendrocytes have a distinct but equally vital role in the central nervous system. Let us examine the development and functions of oligodendrocytes in more detail.

2.5 Oligodendrocytes

Oligodendrocytes (OLGs) are crucial in white matter, forming myelin in the central nervous system to aid electrical signal conduction along axons (Vinet *et al.,* 2010; Michalski and Kothary, 2015). These cells stem from a lineage undergoing proliferation, migration, differentiation, and myelination to insulate axons (Lent *et al.,* 2018). Oligodendrocytes and their precursors not only insulate axons but also provide trophic support to long axons (McTigue and Tripathi, 2008; Nave and Werner, 2014), contribute to white matter angiogenesis signaling (Yuen *et al.,* 2014), support the blood-brain barrier (Seo *et al.,* 2014), and facilitate neuronal circuit information processing (Takahashi *et al.,* 2011; Edgar and Sibille, 2012; von Büdingen *et al.,* 2015). The plasticity of white matter in OLGs is influenced by the transformation of oligodendrocyte progenitor cells (OPCs) into mature OLGs (Boulanger and Messier, 2014), crucial for managing complex cognitive functions like social interactions (Makinodan *et al.,* 2012). Oligodendrocyte dysfunction is significant in neurological and psychiatric conditions, including multiple sclerosis (Vassall *et al.,* 2015) and schizophrenia (Windrem *et al.,* 2017).

Evidence indicates oligodendrocytes are vital for brain function and potential therapeutic targets in pathological situations.

2.5.1 Functions and morphology of oligodendrocytes

Oligodendrocytes are categorized into four types based on morphology: types I and II with small cell bodies, 5-10 processes, and myelinated axons; type III with large cell bodies, 1-2 processes, and myelinated axons; and type IV with large cell bodies without processes, directly positioned over the strand they encase (del Río-Hortega, 1928; Penfield, 1932).

Additional oligodendrocytes with different morphologies have been identified in various brain regions through imaging techniques. The OLGs lineage expresses Olig2, confirming three subtypes: grey matter (satellite or perineuronal), white matter (myelinating cells in fasciculi and tracts), and perivascular oligodendrocytes, observed in adult humans (Tanaka *et al.,* 2005) and developing animals (Tsai *et al.,* 2016). In hippocampus mouse, Vinet *et al.* (2010) identified three developing oligodendrocyte populations: ramified, stellar, and smooth.

In African giant rats, oligodendrocytes are classified into five subtypes: OPC, pre-oligodendrocytes, immature, mature non myelinating oligodendrocytes, mature myelinating oligodendrocytes (Olude *et al.,* 2018). Research in rodents has shown that oligodendrocytes are the most susceptible cells in the CNS due to high-energy requirements for growth and myelin sheath production (Lent e*t al.,* 2018; Valério-Gomes *et al.,* 2018). OLGs are particularly vulnerable to ischemic damage.

2.5.2 Oligodendrogenesis in the avian brain

Olivier *et al.* (2001) found that during embryogenesis, oligodendrocytes in quail-chick chimeras originate basoventrally in the caudal forebrain, while in the forebrain, they arise from alar regions. In chick embryos, pre-oligodendrocytes identified with O4 monoclonal antibody show radial migration. Garcia-Lopez and Martinez (2010) explored the origin and movement of oligodendrocyte precursors in the quail-chick chimera within the diencephalon. Their findings indicated OPCs originate from the medial septum and entopenducular areas. Tangential migration spread throughout the telencephalon in a retroviral study.

O4 antibodies in the spinal cord revealed pre-OLG cells migrating radially to populate the basal mantle in the alar mantle layer; these cells migrate ventrodorsally and mature to form myelin (Miller and Ono, 1998). Ono *et al.* (1998) investigated whether OLG development in the metencephalon follows the same migration pattern as in the spinal cord, using O4 and O1 antibodies. On embryonic day 5, O4 was found in the medial ventricular zone (VZ), by E6 in the lateral pons, and by E7 in the cerebellum. O1 first appeared in the medial region at E8, lateral pons at E10, and cerebellum at E12. O4 and O1 showed similar distribution and migration patterns in these regions (Soula *et al.,* 2001).

In mouse spinal cord, OPCs emerge around E12.5 from the VZ, regulated by Sonic hedgehog (Shh) from the notochord (Pringle *et al.,* 1996). Dorsal ventricular OPCs appear at E15.5 without Shh signaling (Cai *et al.,* 2005), accounting for 20 % of the total OPC population (Tripathi *et al.,* 2011). In the optic nerve, OPCs are in the preoptic region under Shh influence; they migrate and colonize the entire nerve (Merchán *et al.,* 2007; Ravanelli *et al.,* 2018).

Research on oligodendrocytes in birds has focused on their development, with limited studies on the adult brain. Cho *et al.* (1999) explored transferrin-binding protein (TfBP) in the central nervous system (CNS) of adult birds (chickens, pigeons, lovebirds) and mammals (rats, rabbits, and cats). Their findings showed a similar TfBP pattern in birds, with no TfBP-positive oligodendrocytes (OLGs) detected in mammals. An immunoelectron study revealed light and medium OLG subtypes distributed in the perinuclear cytoplasm with delicate processes. Seo *et al.* (2001) examined OLG distribution in adult quail and chicken retinas using anti-TfBP immunohistochemistry. They found a central distribution pattern linked to myelin, with fewer OLGs towards the edges. Three OLG cell types were identified in the ganglion cell layer, each with unique morphology. Cho et al. concluded that TfBP is crucial for iron metabolism in the CNS, and its absence in mammals suggests structural differences among species.

Fischer et al. (2010) identified non-astrocytic inner retinal glia (NIRG) cells in chickens, distinct from Müller glial cells. NIRG cells are involved in retinal metabolism, Müller cell function, and neuronal survival. They express certain astrocyte markers but not GFAP and glutamine synthesis. NIRG cells were found in dog and non-human primate retinas, but not in mice and guinea pigs. Having explored the myelinating functions of oligodendrocytes, we now turn to another critical glial cell type - microglia. As the brain's resident immune cells, microglia have unique roles in the development and maintenance of the central nervous system.

2.6 Microglia

Microglia, the brain's macrophages, are immune glial cells dispersed throughout the central nervous system (Tremblay *et al.,* 2011; Schafer and Stevens, 2015; Sierra *et al.,* 2019), comprising 5 to 20 % of CNS cells, varying between brain and spinal cord (Tay *et al.,* 2016).

Microglial cells differ from astrocytes and oligodendrocytes due to their non-neurogenic origin. They belong to the myeloid/mesenchyme lineage, colonizing the CNS during embryonic development from the yolk sac (Alliot *et al.,* 1999; Ginhoux *et al.,* 2013). Microglia are predominantly found where cell death occurs (Pont-Lezica *et al.,* 2011).

2.6.1 Functions and morphology of microglia

Microglia are crucial during development, selecting and eliminating unnecessary synapses and engaging in synaptic activities (Pont-Lezica *et al.,* 2011; Grigsby *et al.,* 2014; Rose *et al.,* 2017). They release neurotrophic factors and cytokines to modify neuronal circuits and remove excess apoptosis (Tremblay *et al.,* 2010; Marín-Teva *et al.,* 2011; Schafer *et al.,* 2012). These cells have membrane receptors for neurotransmitters and neuroactive molecules (Bieber 2007; Kettenmann and Verkhratsky 2008; Lowry 2012). Research indicates microglial characteristics play a non-immune role in adult neurogenesis (Sierra *et al.,* 2016) and dendritic spine removal (Tremblay et al., 2016). Microglia are implicated in brain sexual differentiation (Bordt *et al.,* 2020), influencing development during high plasticity periods (Kopec *et al.,* 2018), and regulating energy balance (Bobbo *et al.,* 2019). De Luca *et al.* (2020) suggest microglia is essential for controlling satiety, cognition, and food intake. In humans and vertebrates, including birds (Peters, 1991), microglia are "ameboid" during embryonic and postnatal development, and mature into "ramified or resting" microglia (Aloisi, 2001; Mallet *et al.,* 2005).

Nimmerjahn *et al. (*2005) state resting microglia are fully ramified, highly mobile, and constantly monitor surroundings by extending and retracting processes (Sierra *et al.,* 2014).

2.6.2 Microgliogenesis in the avian brain

Pont-Lezica *et al.* (2011) highlighted that the potential roles of microglia during normal development have not been extensively studied. Ramified microglia are found in the plexiform layers of the retina in quails, cats, and rats. But research on their presence in photoreceptors is limited (Navascués *et al.,* 1995; Thanos *et al.,* 1996; Tumosa and Baker, 1996). In some rodent brain areas, microglial activation is observed as part of normal aging. Kunert *et al.* (1999) used immunohistochemical localization of the QH1 antibody to examine microglia migration in juvenile and aged quails (1 year) and pigeons (2-20 years). They found significant rounded microglia predominantly in the periphery of both species (pigeon p 0.002 and quail p 0.01), more pronounced in older birds (pigeon p 0.05 and quail p 0.03). The researchers concluded that microglial activation increases in aged birds and is directly linked to photoreceptor loss in quails.

Another study utilized microglia during chick retinal development to determine if nerve growth factor (NGF) induces cell death by activating the neurotrophic receptor p75. Investigations into microglia distribution in developing and mature quail brains and retinas using QH1 immunohistochemistry showed microglia in the inner and outer plexiform layers throughout the retina and brain (Navascués *et al.,* 1995; Marín-Teva *et al.,* 1999; Jeon *et al.,* 2004; Sánchez-López *et al.,* 2005). A similar distribution pattern is observed in chickens using isolectin B4 staining, mirroring the laminar pattern in mammals (Navascués *et al.,* 1995; Won *et al.,* 2000). Cuadros *et al.* (2006) used the marker HIS-C7 to study microglia distribution in developing and mature chick brains, revealing microglial subtypes throughout the brain and retina, including ramified microglia. Microglia proliferate during the early postnatal period and maintains a stable number in adulthood.

Specific markers like Temii9 and P2ry12 are expressed weeks after birth, facilitating the transition of microglial phenotype to adults in mice (Matcovitch-Natan *et al.,* 2016).

Research on migratory birds has mainly focused on neuron counting and morphology complexity (Mouritsen *et al.,* 2016; Mettke-Hofmann, 2017). Investigations into microglia in avian species have largely been limited to the retina and optic nerves during developmental stages, with scarce information on healthy postnatal brain tissue (Tremblay *et al.,* 2011). A three-dimensional morphological and stereological analysis by Diniz *et al.* (2016) examined two migratory bird species: the semipalmated sandpiper (*C. Pusilla*), which undertakes a five-day non-stop transatlantic flight, and the spotted sandpiper (*A. macularia*), which engages in long-distance overland flights with intermittent stops. The findings indicated that *A. macularia* possesses more microglia and a larger hippocampal formation compared to *C. Pusilla*. However, no differences were found in the total neuron count between the two species. Variations were noted in the morphological complexities of the neuron-to-microglia ratio.

The current study, to the best of my knowledge represents the first study to integrate diverse methodologies and imaging techniques that explored gliogenesis and neurogenesis in relation to age-related changes in the juvenile, subadult and adult male Japanese quail (*Coturnix japonica*) brain.

2.7 Ependymal cells

The complex process of neurogenesis, responsible for the generation of new neurons, and gliogenesis, the formation of glial cells, is fundamental to the development and maintenance of the central nervous system (Finkel et al., 2021). Within this intricate landscape, ependymal cells, specialized epithelial cells lining the brain ventricles, have emerged as a subject of significant interest due to their multifaceted roles.

Particularly in the context of neural stem cell niches (Abdi et al., 2018). These cells are derived from radial glial cells during embryonic development, which are critical for the formation of the brain's complex architecture (Angelopoulos et al., 2022). Previous research has indicated that both neural stem cells and ependymal cells share a common lineage, originating from radial glial cells (Zheng et al., 2025). This shared developmental origin has led to extensive investigations into the potential of ependymal cells to function as neural stem cells themselves or to significantly influence neurogenic and gliogenic processes in the adult brain (Coskun et al., 2008., Ioannidis et al., 2021). However, the precise role of ependymal cells in adult neurogenesis and gliogenesis remains a subject of ongoing debate and intensive research, with studies presenting conflicting evidence regarding their stem cell potential and contribution to neural repair following injury (Muthusamy et al., 2018., Kriegstein & Álvarez-Buylla, 2009).

This review aims to synthesize current knowledge regarding ependymal cell biology, their developmental origins, and their involvement in neurogenic and gliogenic processes, specifically examining the evidence supporting and refuting their role as bona fide neural stem cells.

2.7.1 Ependymal cells and neurogenesis

The structural and functional characteristics of ependymal cells in neurogenic regions are crucial components of neurogenic niches, exhibit unique morphological features that contribute to their functional roles in the central nervous system (Capilla-Gonzalez et al., 2014; Quaresima et al., 2022; Marcy et al., 2023). In the ventricular-subventricular zone (V-SVZ), a key neurogenic niche, ependymal cells originate from radial glial cells during postnatal development and form a structural and trophic component alongside neural stem cells (NSCs) (Kyrousi et al., 2017).

Morphologically, these cells are multiciliated, which is essential for facilitating cerebrospinal fluid (CSF) flow and possibly sensing environmental changes within the ventricle (Kyrousi et al., 2017; Spassky et al., 2005 Kyrousi et al., 2017; Lalioti et al., 2019 ; Macdonald et al., 2021; Chae et al., 2023).

The differentiation of ependymal cells from radial glial cells is regulated by signaling pathways that involve transcription factors such as FoxJ1(Ogino et al., 2016 ; Jacquet et al., 2009; Macdonald et al., 2021; Chae et al., 2023). FoxJ1 expression in radial glial cells is necessary for their differentiation into multiciliated ependymal cells, which form part of the postnatal neural stem cell niche in the lateral ventricles (Jacquet et al., 2009). These multiciliated ependymal cells are characterized by their distinctive pinwheel structure, where NSCs form the core and ependymal cells provide a supportive lining (Zhang et al., 2014). Ependymal cells also play a reactive role following spinal cord injuries (Paniagua-Torija et al., 2018; Chevreau et al., 2021; Stenudd et al., 2022; Yue et al., 2024). They proliferate and contribute to the formation of a glial scar, utilizing gap junctions and connexin signaling to facilitate communication and structural integrity (Fabbiani et al., 2020). In the case of such injuries, ependymal cells can revert to a more progenitor-like state, which is indicative of their latent regenerative potential (Taupin, 2006). In summary, the morphological features of ependymal cells, such as multiciliation and their unique structural positioning in neurogenic niches, are illustrative of their multifaceted role in both supporting stem cell niches and responding to injury. Their differentiation from radial glial cells is tightly regulated by genetic and molecular pathways, (Jurisch-Yaksi et al., 2020; Miranda-Negrón & García-Arrarás, 2022; Laddach et al., 2023) underscoring their importance in maintaining neuroplasticity and regenerative potential in the central

nervous system (Deng et al., 2022; Radoszkiewicz et al., 2024; Xie & Li., 2024; Tataranu & Rizea, 2025).

2.8 The Japanese quail (*Coturnix japonica*) as a model for gliogenesis research

The Japanese quail (*Coturnix japonica*) is a precocial songbird classified under the order Galliformes and the family Phasianidae (Minvielle, 2004). It is considered distinct from the common quail (Huss *et al.,* 2008; Sophie *et al.,* 2010). These small, domesticated birds hold significant economic value (Owen and Dike, 2013; Dauda *et al.,* 2014) due to their fast growth, brief gestation period, and high reproductive rates (Afrose *et al.,* 2011; Owen and Dike, 2013). Beyond their social and economic importance, Japanese quails serve as valuable research models for investigating various biological processes (Padgett and Ivey, 1959; Huss *et al.,* 2008), including immunology, endocrinology, reproductive events, and aging (Minvielle, 2004; Holmes and Ottinger, 2003; Ottinger *et al.,* 2004; Minvielle *et al.,* 1992). They are also used in studies of brain development (Marasco *et al.,* 2016), neuroanatomy, and neuronal function (Castagna *et al.,* 2003), as well as in ontogenetic development models (Mills *et al.,* 1997).



Figure 2.4: Wildtype plumage of the male adult Japanese quail (Coturnix *Japonica)* (Baer, 2015)

In a recent study, Nkomozepi and Ihunwo (2018) investigated how the age of male Japanese quail (*Coturnix japonica*) after hatching affects neurogenic activities in their brains. They used immunohistochemical staining techniques to detect proliferating cell nuclear antigen (PCNA) and doublecortin (DCX). The research revealed that the age post-hatching has an impact on the distribution of neurogenic markers across different brain regions in the Japanese quail, including the olfactory and telencephalic ventricles, telencephalon, cerebellum, diencephalon, mesencephalon, and optic tectum (Nkomozepi *et al.,* 2018). The Japanese quail has been a valuable model for studying the development and function of the nervous system.

Considering the presence of neurogenic cells in adulthood, it is fascinating to explore the possibility of ongoing gliogenic and neurogenic processes in their brains during this life stage. While adult neurogenesis has been extensively examined across various species, there remains a notable scarcity of literature concerning age-related adult gliogenesis in avian species.

The current study, to the best of my knowledge was the first to focused on investigating the process of gliogenesis and neurogenesis within the brains of male Japanese quails at juvenile, subadult, and adult stages

The study characterized gliogenesis through the identification of neural stem cells, assessment of proliferation activity, differentiation, lineage patterns of resultant cells, and the potential co-existence of gliogenesis with neurogenesis within a specific brain region. Additionally, the ultrastructure of glia, neurons, and mitochondria were also examined.

CHAPTER THREE: Materials and Methods

3.1 Experimental animals and ethical consideration

Thirty (30) male Japanese quails (Coturnix japonica), ranging in age from 3 to 12 weeks, were categorized into three (3) distinct age groups: juveniles (3-5 weeks old), subadults (6-8 weeks old), and adults (9-12 weeks old) (Nkomezepi et al., 2018). These quails were sourced from A & Z Aviaries in Durban, South Africa, via the Central Animal Services (CAS) at the University of Witwatersrand, Johannesburg. The animals were housed in separate plastic brooder cages with unlimited access to food and water. Body weights were recorded weekly. The light cycle was maintained at 16 h of light and 8 h of darkness, with the temperature set to 25 °C. These age categories were chosen to represent juveniles (3 – 5 weeks, sexually immature), subadults (6 – 8 weeks, sexually mature), and adults (9 – 12 weeks, sexually active), respectively. All animal protocols were performed in accordance with the University of Witwatersrand Animal Ethics Screening Committee (ASEC NO: 2018/11/59/A), which is consistent with the National Institute of Health (NIH) guidelines for the care and use of animals in scientific experimentation.

3.2 Tissue collection and processing

Birds from the juvenile, subadult, and adult experimental groups were euthanized at 4, 6, and 12 weeks, respectively. Intramuscular injection of heparin (1 ml each) to prevent coagulation. Five minutes later, the birds were dead after sodium pentobarbital (0.3-1 ml/kg, i.p.) was administered. The birds underwent open chest intracardial perfusion, starting with a rinse of 0.9 % saline solution, followed by 4 % paraformaldehyde in 0.1 M phosphate buffer solution. The brain was extracted from the skull and post-fixed overnight (24 h) in 4 % paraformaldehyde in 0.1 M PB at 4 °C and then equilibrated in 30 % sucrose in 0.1 M PB at 4 °C for three days.

The tissues were subsequently moved to an antifreeze solution (comprising 30 % glycerol, 30 % ethylene glycol, 30 % distilled water, and 10 % 0.244 M PB), maintained at 4°C until equilibrium was reached, and stored in a freezer at −20°C. Prior to sectioning, the brains were equilibrated in 30 % sucrose in 0.1 M PB at 4 °C. The brains were then frozen using crushed dry ice and sliced into 50 µm thick sections using a freezing microtome and cryostat. The sections were cut in the coronal plane in one of the eight series. Serial sections were placed in an antifreeze solution and stored at -20 °C for future immunohistochemical analysis. Frozen 1 in 8 serial coronal 50 µm thick sections were cut on a cryostat (Thermo Scientific HM 525 NX) and freezing microtome for free floating method of immunohistochemistry

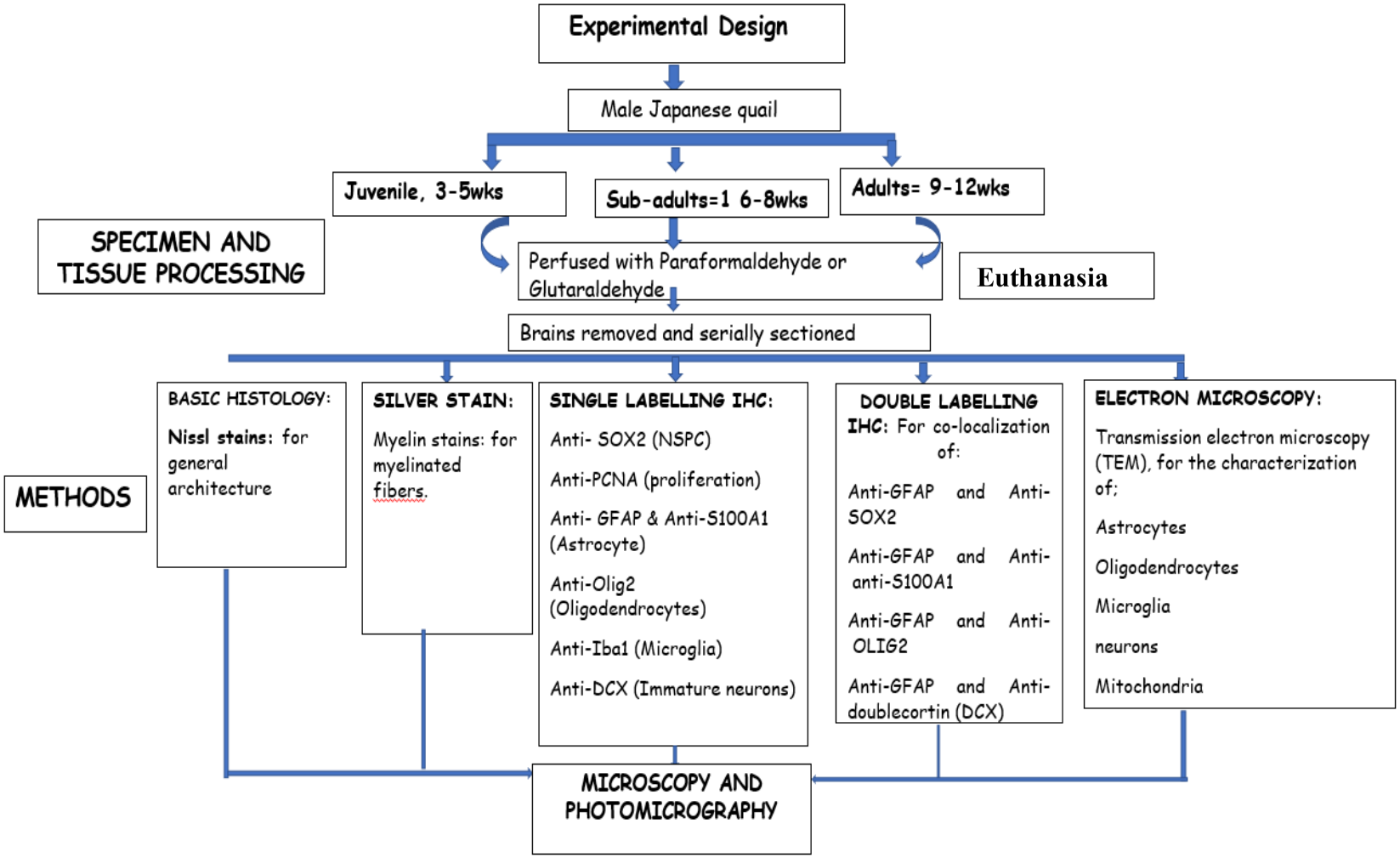


Figure 3.1: Flow diagram showing the experimental design

3.3 Histological staining techniques

3.3.1 Nissl stain method

The first series of eight coronal serial sections was used for Nissl staining. Nissl sections from the juvenile, subadult, and adult experimental groups were mounted on glass slides coated with 0.5 % gelatin and allowed to dry overnight in an oven at 36 °C. Subsequently, the dried sections were cleared overnight in a solution composed of equal parts of chloroform and 100 % alcohol. The sections were then immersed in a series of graded ethanol solutions for rehydration (100 %, 100 % for 5 minutes each, 95 %, 70 %, 50 % for 2 minutes each). The sections were stained with 1 % cresyl violet (1 g in 100 ml distilled water) for 1 minute. The staining process was carefully controlled to ensure optimal contrast and clarity and dehydrated using a series of alcohol (50 %, 70 %, 95 % for 2 minutes each, 100 %, 100 % for 5 minutes each) and xylene treatments twice for 5 minutes each. The sections were covered with entellen and allowed to dry. Nissl-stained sections were used to describe the internal anatomy of the Japanese quail brain. Several internal brain structures were identifiable in the nissl stained regions, delineating the areas that exhibited defined borders of the brain.

3.3.2 Myelin silver stain for myelinated fibers

The second series of sections designated for myelin staining were stored at refrigeration temperatures of 4 °C for a duration of two weeks in 5 % formalin solution. Subsequently, these sections were mounted on glass slides coated with 1.0 % gelatin and allowed to dry for two days. The staining process employed a modified silver staining method, as described by Gallyas (1979). The mounted sections were rehydrated in distilled water twice for a duration of three minutes.

They were then incubated in a 2:1 mixture of pyridine and acetic anhydride for two hours (2 hrs.), followed by immersion in 50 % and 25 % ethanol for three minutes each. This was achieved by treatment with acetic acid solutions of varying concentrations (0.05 %, 0.1 %, and 0.5 %) for three minutes each. The sections were then exposed to a silver solution composed of 0.76 g ammonium nitrate, 0.78 g silver nitrate, and 4 % NaOH dissolved in distilled water for 45 minutes, followed by a 10 minute rinse in 0.5 % acetic acid. During the development phase, the sections were placed in a mixture of solutions A, B, and C at 20 °C. Solution A contained 50 g sodium carbonate, Solution B comprised 1.9 g ammonium nitrate, 2 g silver nitrate, and 10 g silicotungstic acid, and Solution C included 1.9 g ammonium nitrate, 2 g silver nitrate, 10 g silicotungstic acid, and 6.6 ml of 37 % formalin, all dissolved in distilled water for 45 minutes.

The sections were then rinsed with 0.05 % acetic acid for one minute. In the differential step, sections were briefly immersed in 0.2% potassium ferricyanide to enhance stain contrast, followed by a 1 minute rinse in distilled water. The stained sections were fixed in 0.5 % sodium thiosulfate, rinsed three times for one minute each in distilled water, dehydrated using a series of alcohols (50 %, 70 %, 95 % 100 % for two minutes each, , 100 % for 5 minutes each), followed by xylene three times for 5 minutes each, and finally covered with entellen.

3.4 Immunohistochemistry (IHC) methods

Table 3.1 Summary of primary and secondary antibodies, conjugated fluorochromes, serums and mounting media used for immunohistochemistry

SOX2	Sex determining SRY-related high mobile gene 2 (SOX2) antibody
PCNA	Proliferating cell nuclear antigen
GFAP:	Glial fibrillary acidic protein
S100A1:	S100A1 protein
OLIG2:	Oligodendrocyte lineage transcription factor 2
IBA-1:	Ionized calcium binding adopter molecule 1
DCX:	Doublecortin
ABC Kit:	Avidin biotin complex kit
DAB:	Diaminobenzidine
BSA:	Bovine serum albumin

S/N	Primary antibodies	Dilution	Host specie clone	Company	Cat number
1	SOX2	1:1000	Rabbit polyclonal	Abcam	ab 97959
2	PCNA (Pc-10)	1:1000	Mouse monoclonal	Abcam	ab 29
3	GFAP	1:1000	Mouse monoclonal	Millipore sigmaAldrich	MAB 360 clone G-A.5
4	S100	1:500	Rabbit polyclonal	Abcam	ab 868
5	OLIG2	1:1000	Rabbit polyclonal	Millipore	AB 9610
6	IBA-1	1:800	Mouse monoclonal	Sigma-Aldrich	SAB 27023
7	DCX	1:1000	Rabbit polyclonal	Abcam	ab 18723
Secondary antibodies for single labelling immunohistochemistry					
8	Goat anti mouse	1:1000		Vectors	A1- 9200BA-9200
9	Goat anti rabbit	1:1000		Vectors	BA-1000
10	ABC kit			Vectors	PK-6100
11	Normal goat serum				
12	DAB			Sigma	SHBF 9794
13	BSA				
Secondary antibodies for double labelling immunohistochemistry					
14	Goat anti rabbit Alexa fluor (488)	1:1500		Abcam	ab 150077
15	Goat anti mouse Alexa fluor (647)	1:1500		Abcam	ab 150115
16	Dapi	1:10000		Invitrogen molecular probes	D 1306
17	Fluor mount			Vectors	H-1400

3.4.1 Free floating method for immunohistochemistry (IHC)

3.4.1.1 Single labelling immunohistochemistry for SOX2, PCNA, GFAP, S100A1, OLIG2, IBA-1 and DCX

Brain sections were first treated for 30min with a mixture of an endogenous peroxidase inhibitor (49.2 % methanol: 49.2 % 0.1 M PB: 1.6 % of 30 % H_2O_2). They were then rinsed three times with 0.1 M PB for 10 min each. Subsequently, the sections were placed in blocking buffer solution for two hours at room temperature. This solution contained 5 % normal goat serum (NGS), 2 % bovine serum albumin (BSA), and 0.25 % Triton X-100 in 0.1 M PB. The sections were mixed with rabbit polyclonal antibodies: Sex determining SRY-related high mobile gene 2 (SOX2) antibody(SOX2) (1:1000 dilution of ab 97959, Abcam, rabbit polyclonal), proliferating cell nuclear antigen PCNA (Pc-10) (1:1000 dilution of ab 29, Abcam, mouse monoclonal), glial fibrillary acidic protein GFAP (1:1000 dilution of MAB 360 clone G-A.5, Millipore Sigma Aldrich, Mouse monoclonal), S100 protein A1 S100A1 (1:500 dilution of ab 868, Abcam, Rabbit polyclonal), Oligodendrocyte lineage transcription factor 2 OLIG2 (1:1000 dilution of AB 9610, Millipore, Rabbit polyclonal), ionized calcium binding adopter molecule 1 IBA-1 ((1:800, dilution of SAB 27023, Sigma-Aldrich, Mouse monoclonal), and doublecortin DCX (1:1000 dilution of ab 18723, Abcam, Rabbit polyclonal) in blocking buffer BSA () and incubated at 4 °C for 48 h with light shaking. Three 10 minutes rinses followed the primary antibody incubation in 0.1 M PB.

Next, the sections were placed in a secondary antibody solution containing biotinylated goat-anti-rabbit IgG, BA1000, (diluted 1:1000), biotinylated goat anti-mouse, A1-9200BA-9200, Goat anti-rabbit, BA-1000, Avidin biotin complex kit, and PK-6100, all from (Vector laboratories, Burlingame, California, USA in a mixture of 2 % NGS and 2 % BSA in 0.1 M PB). They were left at room temperature for 2 h with gentle shaking. Three 10 minutes rinses in 0.1 M PB were performed first, and then the sections were placed in an avidin-biotin solution (AB complex) (at a concentration of 1:125, Vector Labs) for one hour. After that, three 10 minutes rinses each in 0.1 M PB were performed again.

The sections were then placed in a solution of 0.05 % diaminobenzidine (DAB) in 0.1 M PB for 5 min, then 3.3 µl of 30 % hydrogen peroxide was added to each 1 ml solution. The chromatic precipitation was visually monitored using a low-power stereomicroscope until the background stain reached a level that would permit precise architectonic matching to the Nissl sections without masking the immunopositive structures. Sections were then placed in 0.1 M PB to stop the DAB reaction, followed by two more rinses for 10 minutes each in 0.1 M PB and then the reaction was terminated. The sections were then mounted on 0.5 % gelatine-coated glass slides, dried overnight, dehydrated in a graded series of alcohol (50 %, 70 %, 95 % for 2 minutes each, 100 %, 100 % for 5 minutes each), cleared in xylene, and cover slipped using Depex. To test for non-specific staining in the immunohistochemical protocol, primary antibodies were omitted from selected areas, which resulted in no staining of the sections.

3.4.2 Double labelling immunohistochemistry method

Free-floating coronal serial sections were washed with phosphate buffer saline (PBS) and blocked with 10 % normal goat serum (NGS) in PBS. The primary antibodies were diluted in PBS containing 0.3 % triton x and 10 % NGS. Immunostained sections were treated with GFAP and SOX2 for neural stem progenitor cells, GFAP and S100 for neural stem progenitor cells and astrogliogenesis, GFAP and OLIG2 for neural stem progenitor cells and oligodendrogenesis, GFAF and DCX for neural stem progenitor cells and neurogenesis.

Subsequently, the sections were incubated with primary antibodies in blocking buffer for 48 h at 4 °C with gentle agitation. The sections underwent three 15 minutes rinses. The sections were incubated with a secondary antibody solution of biotinylated goat anti-mouse (Alexa Fluor conjugated (647) red, ab 150115 Abcam) and biotinylated goat anti-rabbit (Alexa Fluor conjugated (488) green, ab 150077, Abcam) at a 1:1000 dilution and incubated at room temperature in the dark for two hours. Sections were washed 4 times in 15 minutes, incubated with DAPI (1:10000) for 5 min, and washed four times for 15 min. Mount with fluoroshield and store in the dark, in the fridge until viewing.

3.5 Transmission electron microscopy (TEM) method

Three (3) Japanese quail (*Coturnix japonica*) at juvenile, subadult, and adult males were procured and humanely euthanized in accordance with the ethical guidelines for animal research. The brains were excised and fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2–7.3) for 4 h. The sections were subsequently washed in phosphate buffer (3 changes of 10 min each) and leave overnight in fridge. Post-fixation was performed in 1 % osmium tetroxide in phosphate buffer for 1 h.

The sections were washed in phosphate buffer with gentle agitation for 5 minutes and stored in 70 % ethanol at 4 °C. Dehydration was performed at room temperature using absolute ethanol (70 % ethanol for 15 minutes, 95 % ethanol for 10 minutes, and absolute ethanol for 2 × 10 minutes). Clearing was achieved using propylene oxide for 2 × 10 minutes. The tissue sections were infiltrated and embedded in propylene oxide: resin (3:1) for 30 minutes, followed by propylene oxide: resin (1:1) for 30 minutes, and propylene oxide: resin (1:3) for 30 minutes. The sections were then placed in resin alone (in a dry container) overnight. An ultramicrotome was employed to obtain a semi-thin section (1 – 1.5 μm), which were stained with sodium borax and toluidine blue. The areas of interest were then examined using a light microscope (Axiostar Plus, Zeiss, South Africa).

Ultrathin serial sections (60 nm) were obtained and contrasted using lead citrate and uranyl acetate. Six to nine sections from five grids were analyzed for each specimen, and electron micrographs were captured using a transmission electron microscope (Fei Tecnai T12, TEM).

3.6 Microscopic analysis for histology and immunohistochemistry

To compare brain structures, a low-power microscope was employed to examine brain tissue sections that were stained with nissl and myelin silver stain, subjected to immunohistochemical analyses for SOX2, PCNA, GFAP, S100A1, OLIG2, IBA-1, and DCX. Panoramic photomicrographs were obtained using an inverted stereomicroscope (Nikon SMZ 1500) and captured using a DS-Fi 1 Nikon camera (Japan). These images offer a comprehensive view of the sections, allowing for the clear identification of brain regions marked by antibodies. The structures were identified and labeled according to the quail and chicken brain atlas (Kuenzel, 1988; Puelles, 2007; Panzica, 1995).

The nomenclature is based on the Avian Brain Forum (Reiner et al., 2004). The relative densities and distribution of antibody expression across various brain regions were visually assessed and documented on a scale indicating high (+++), moderate (++), low (+) and absent (-) levels.

High-resolution digital photomicrographs were captured with camera 208 (Axio Cam HRc, Zeiss South Africa) mounted on the light (Axioskop 2 plus, Zeiss, South Africa) and operating on the Zen blue 3.12 software. Fluorescence images from the double labelling IHC were obtained using a Zeiss laser scanning confocal microscope (LSM 780), operated with Zen Blue 3.12 software. No pixilation was applied, aside from adjustments to contrast, brightness, and levels, using Photoscope X version.

3.7 Electron microscopic analysis

The ultrastructure of astrocytes, oligodendrocytes, microglia, neurons, and mitochondria were captured with a Fei Tecna T12 transmission electron microscope. The images were automatically scaled at 500 nm, 1000 nm, and 2000 nm.

3.8 Statistical analysis

Results are shown as the mean and standard error of the Mean (SEM). Version 9 of GraphPad Prism was used for the statistical analysis (GraphPad Software, CA, USA). The Shapiro-Wilk test was used to determine whether the data was normal (normality test). One-Way analysis of variance (ANOVA) was performed to compare the body weight and brain weight among juvenile, subadult and adult quail birds. After a meaningful P value was reached. The threshold for statistical significance was set at ($P < 0.0001$) and said to be statistically significant. Furthermore, linear regression was used for correlation between body and brain weight using Pearson correlation.

CHAPTER FOUR: Results

4.1 Cytoarchitecture and myelinated fibers in the Japanese quail brain

The avian brain, particularly that of a model organism such as the Japanese quail (*Coturnix japonica*), can be used to study the spatial organization of neuroanatomy and developmental changes in neural and glial populations. The aim of this study was to provide a detailed microscopic description of the regional subdivisions and distribution of glial cells and neurons in the male Japanese quail brain at juvenile, subadult, and adult stages. Cytoarchitecture and myelination patterns were assessed in serially sectioned brain specimens utilizing Nissl and Myelin silver stains. I also investigated the relationship between bird weight and brain weight, providing insights into how these two variables correlate. My results illustrated differences in neuronal density, the distribution of glia, and myelination across neuroanatomical structures, capturing the dynamic development of avian brain. These insights not only expand knowledge of avian neuroanatomy, evolutionary adaptations and their cognitive abilities. It also serves as a foundation for future comparative work in this area across species.

4.1.1 Relationship between bird weight and brain weight in the Japanese quail bird

Table 4.1: Mean bird weight and brain weight of juvenile, subadult and adult quail birds

Parameters	Juvenile	Subadult	Adult	F	P-Value
Bird Weight	131.1±5.30	221.6±6.44[a]	298.6±13.39[ab]	84.66	<0.0001
Brain Weight	0.84±0.02	0.93±0.01[a]	0.97±0.01[a]	16.05	<0.0001

Data is expressed as mean standard error of mean: Mean ± SEM and analyzed by One-way analysis of variance ANOVA, n = 7, a= significantly different from Juvenile; b=significantly different from Subadult ; ** = P = 0.003, **** = P<0.0001; n = 7. Error bars correspond to the standard error of the mean (SEM).

4.1.2 Bird weight in the juvenile, subadult and adult Japanese quail bird

A One-way Analysis of Variance was performed to compare the body weight among juvenile, subadult and adult quail (Figure 4.1). The result showed a significant effect of age on body weight $F(2,18) = 84.66$, $P < 0.0001$. There was a significant increase ($P < 0.0001$) in bird weight of subadult (221.6±6.44), and adult (298.6±13.39) quails compared to the juvenile quails (131.1±5.30) (Table 4.1). Also, adult quails had significantly greater body weight compared to subadult quails ($P < 0.0001$) as shown below.

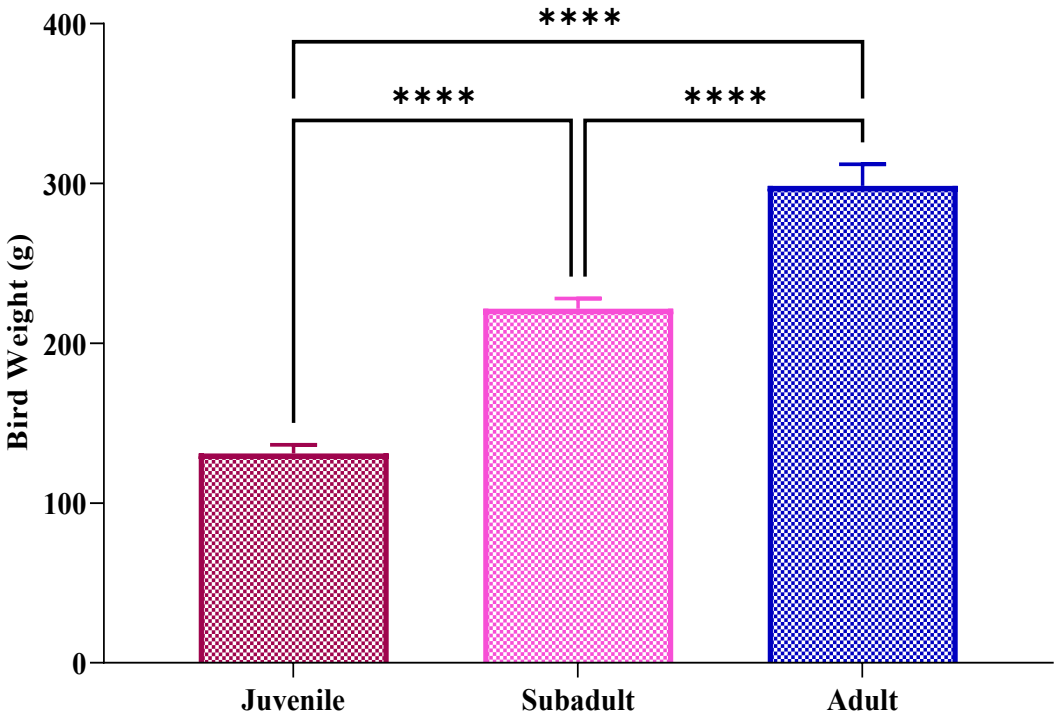


Figure 4.1: Mean bird weight juvenile, subadult and adult quail birds. Data are expressed as Mean ± SEM analyzed by One-way ANOVA; **** = P<0.0001; n – 7. Error bars correspond to standard error of the mean (SEM).

4.1.3 Brain weight in the juvenile, subadult and adult Japanese quail bird

A One-way Analysis of Variance was performed to compare the body weight among juvenile, subadult and adult quails (Figure 4.2). The result showed a significant effect of age on brain weight $F(2,18) = 16.05$, $P < 0.0001$. There was a significant increase ($P < 0.0001$) in brain weight of subadult (0.93±0.01) and adult (0.97±0.01) quails compared to the juvenile quails (0.84±0.02) (Table 4.1). However, there was no significant difference in the brain weight of adult quails compared to subadult quails ($P = 0.26$) as shown below.

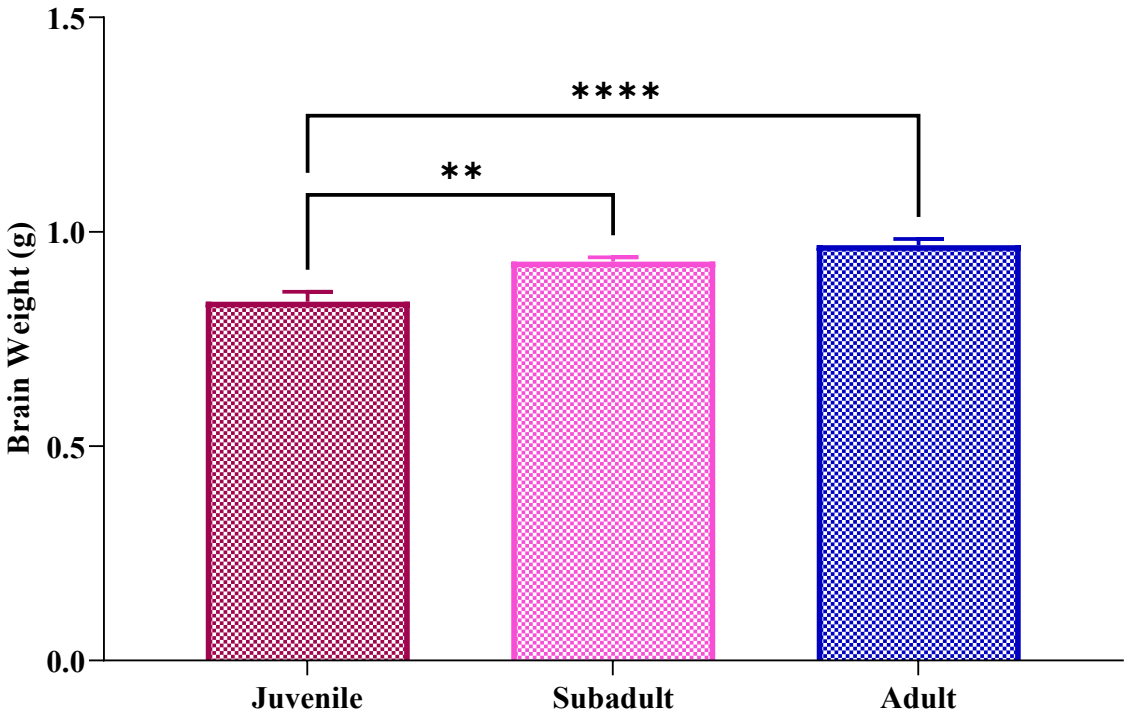


Figure 4.2: Mean brain weight of juvenile, subadult and adult quail birds. Data are expressed as Mean ± SEM analyzed by One- way ANOVA; **** = P<0.0001; n = 7**.** Error bars correspond to the standard error of the mean (SEM).

4.1.4 Correlation analysis between bird and brain weight in the juvenile, subadult and adult quail.

The linear regression analysis reveals a statistically significant positive correlation between bird weight and brain weight. Pearson correlation was used, the upward-sloping red regression line indicates that as bird weight increases, brain weight also tends to increase (Figure 4.3). The pink shaded region around the regression line represents the confidence interval, which provides a range of values within which the true regression line is likely to fall. This interval is crucial for understanding the reliability of the regression estimate and indicates variability in brain weight predictions based on bird weight.

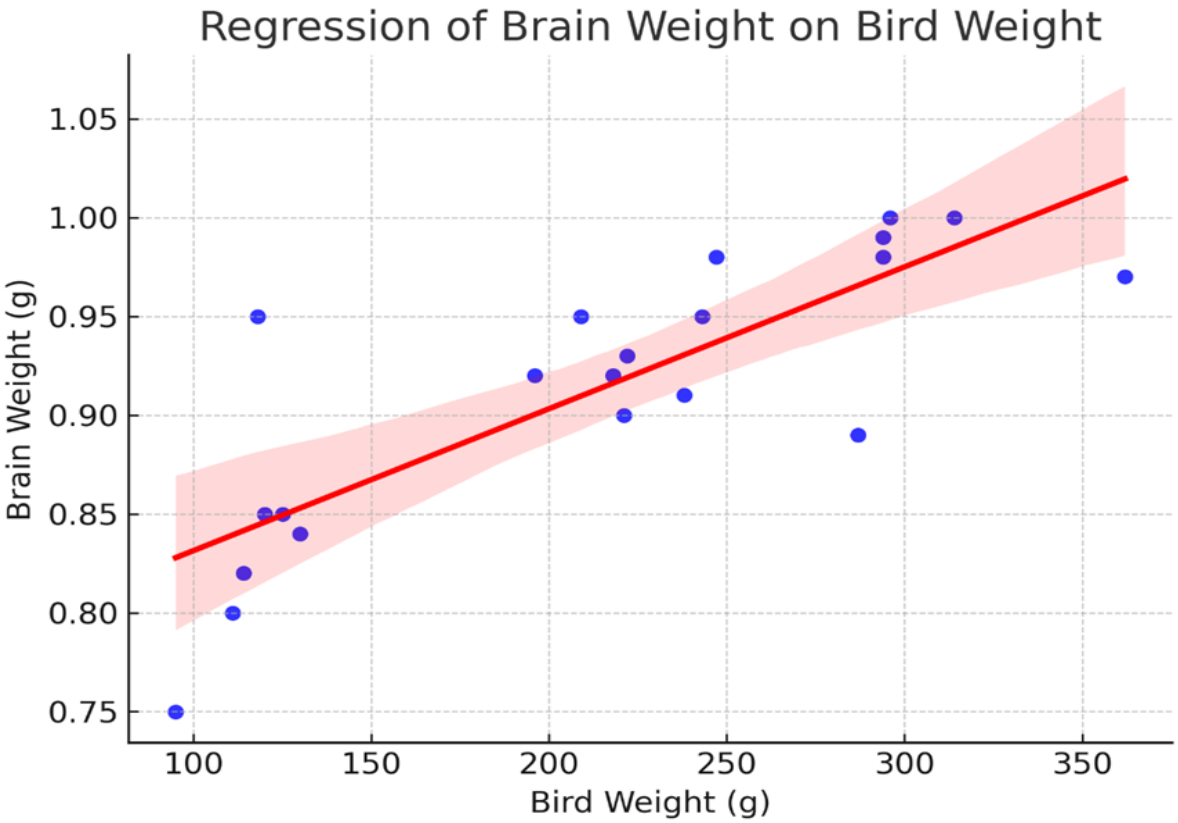


Figure 4.3. Regression plot showed a strong positive relationship between bird weight and brain weight. The red line represents the best-fit regression line, indicating that as bird weight increases, brain weight also increases.

4.2 Myelinated fibers identified by myelin silver stain in the quail brain

A collection of myelin-stained juvenile, subadult and adult brain sections presented in the coronal plane were obtained presented each 50 μm apart. This series demonstrates the cytoarchitecture and myeloarchitectonic structure of the japanese quail brain. Alternating sections were stained with either Nissl (Cresyl violet) or myelin (Gallyas silver) stains, respectively (Figure 4.4- 4.7).

The Panoramic photomicrographs were obtained using an inverted stereomicroscope (Nikon SMZ 1500) and captured using a DS-Fi 1 Nikon camera (Japan). The entire brain sections derived from the juvenile quail brains were between (145-178) sections, subadult (183-218) and adult (188-224) serial sections and a scale of 1mm

Table 4.2: Summary table for the distribution of myelin stain in the quail brain

Brain Region	**Juvenile**	**Subadult**	**Adult**
	3 weeks	**6 weeks**	**12 weeks**
LV	++	+	+
FPL	++	+	+/++
GP	++	+	+
QF	++	++	++
HP	+	+	
VZ	+++	+++	-/+
TSM	+	+	++
CA	++	+	
Optic tectum	++	+	+
OT	++/+++	++	+
SO	++/+++	++	++
SAC	+++	++	
SGF	+++		
RT	++	+	+
Tectal ventricle	+++	++	++
Nerve nuclie	+++	+++	++
NUT	+++	+++	++
MLad	+++	+++	++
Pial surface	++		
Mesencephalon	++	+++	+++
FML	++	+++	+++
Gct	++	+++	+++
OMV	+++		+++
Nbor	+		
PCL	++/+++	+++	
Cerebellum	+++		
CBI	++		
CBL	+	+++	
VEM	+++	+++	+++
VED	+++		+++
Epithalamus		++	
Medial and lateral habenula		+	
MCL		+	
SLU		++	
MCC	+++		
CP	+		

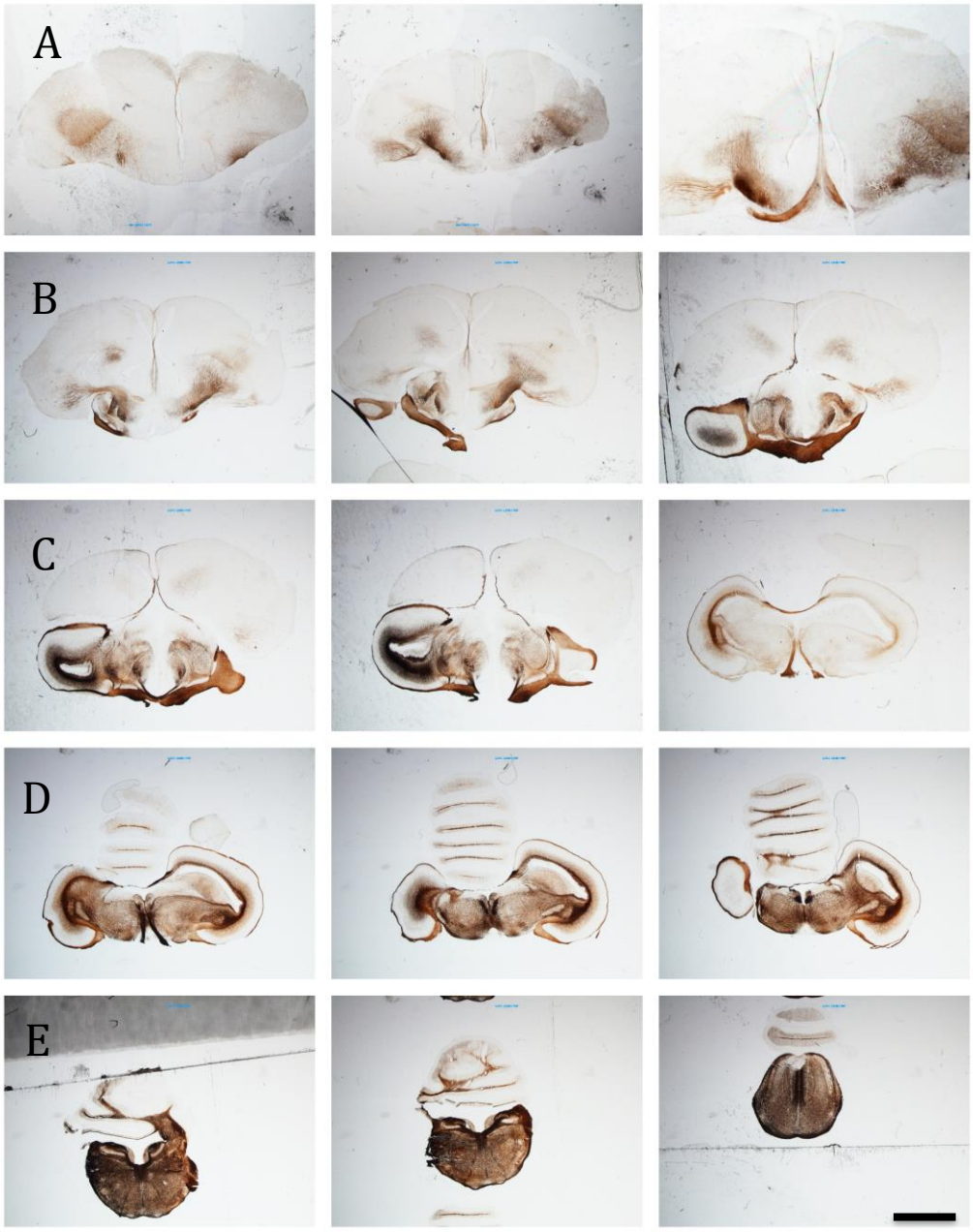


Figure 4.4: A collection of the distribution of myelinated fibers in the brain of the juvenile bird. Panoramic view **(A)**: pallium, **(B)**: diencephalon **(C):** mesencephalon and optic tectum **(D):** cerebellum and **(E)**: pons and medulla. Scale bar: 1mm applies to all.



Figure 4.5: A collection of the distribution of myelinated fibers in the brain of the subadult bird. Panoramic view **(A)**: pallium, **(B**): diencephalon **(C):** mesencephalon and optic tectum **(D):** cerebellum, pons and medulla. Scale bar: 1mm applies to all



Figure 4.6: A collection of the distribution of myelinated fibers in the brain of the adult bird. Panoramic view **(A)**: pallium, **(B**): diencephalon **(C):** mesencephalon and optic tectum **(D):** cerebellum: pons and medulla. Scale bar: 1mm applies to all

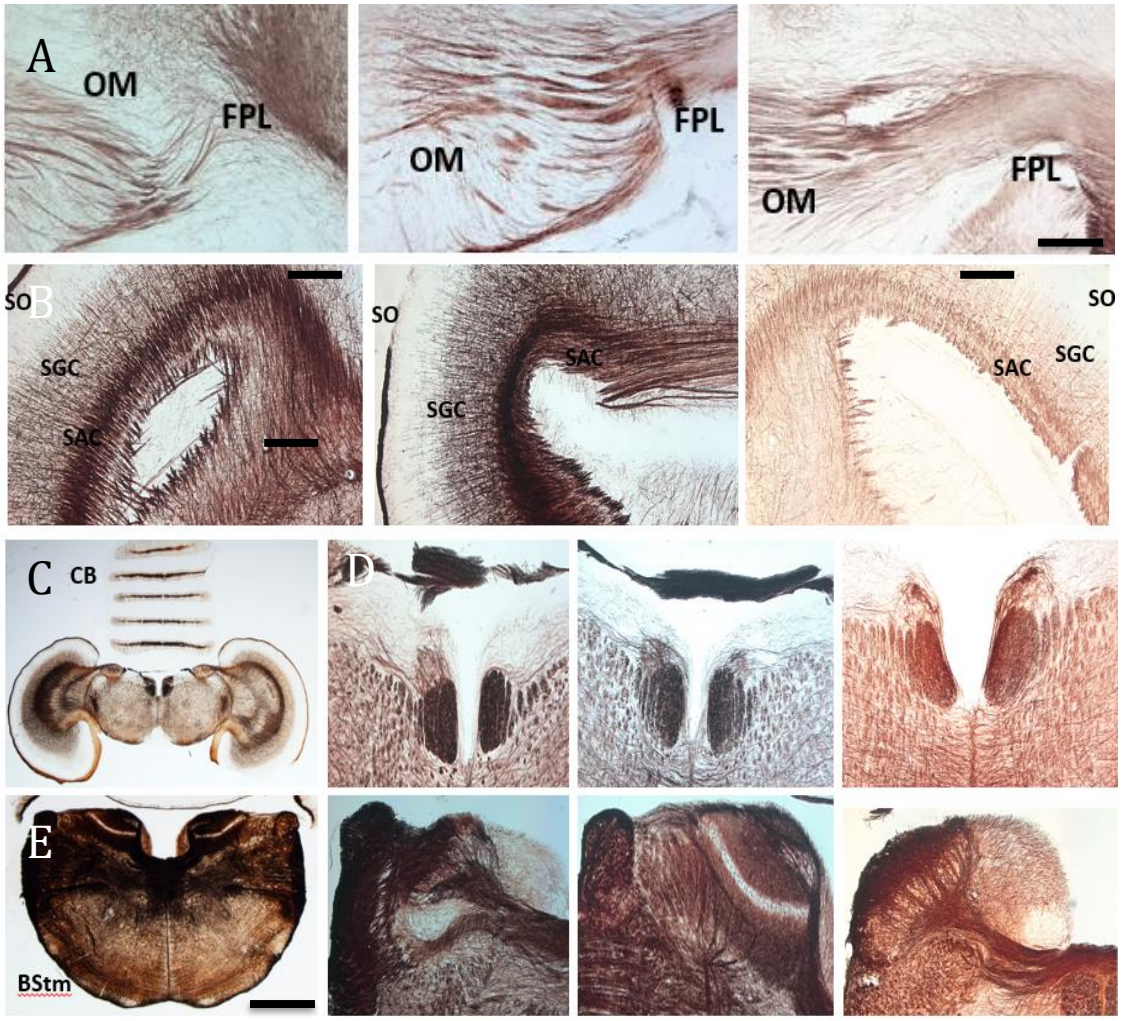


Figure 4.7. Panoramic photomicrography: a collection of myelin silver stains in the juvenile quail brain revealing the myelinated fibers and myeloarchitectonic structures. **(A)** Moderate densities of the axonal projection were observed in the ventricular wall and subpallium but absent in the core pallium. In the diencephalon high density were observed in the optic tract and optic chiasma **(B).** The mesencephalic area **(C)** showed prominent connections of high and moderate myelinated fibers. In the optic tectal region, SO, SGFS, tectal ventricle and 3rd nerve nuclei showed a high density of myelin stain as compared to the core area **(D)**: cerebellar white matter exhibits a high density of myelinated fibers. In **(E),** the pons and medulla showed a high density of myelinated fibers. Scale bar: A, B, D 500µm; C, E 1mm.

4.3 Distribution of neural stem and progenitor cells identified by SOX2 in the quail brain

General observations

From this study, I observed the pattern of expression of SOX2-ir cells at the periphery of the telencephalon but devoid at the core pallial regions except for the hyperpallium and entopallium. High density of SOX2-ir was expressed in the ventricular zone of the lateral ventricle, hippocampus, habenula, hypothalamus and thalamus of the diencephalon, optic tectum, optic ventricle and the cerebellum. SOX2-ir cells were of moderate density in the third and fourth ventricles and low in the brain pons and medulla (Table 4.2). Piamater has a very high density of SOX-ir surrounding the entire brain with some cells spreading inwards towards the pallium. SOX-ir from the caudal area of the pial surface migrates towards anterior and posterior commissure, (CA), piriform cortex (CP) and the optic tectum (Table 4.2). An important and unique observation was in the diencephalon around the preoptic area, they exhibit a larger cell body having a unipolar and or bipolar processes which could be (radial glial-like cells), a high-density cytoplasm and occasionally smaller cells surround the cytoplasm (Figure 4.8). In the diencephalon, highest density is located at the periventricular area along its rostrocaudal axis. As it moves towards the ventral part it spreads dorsally into the adjacent nucleus. Similarly, this pattern is seen at the outer lining of the SO of the optic tectum and outer molecular layer of the cerebellum (CB) and purkinje cell layer (PcL). It was observed that the juvenile and subadult brains have the highest density of SOX2-ir cells compared to the adult birds.

4.3.2 Telencephalon

The pial surface exhibits a high density of SOX2 immunoreactive cells (SOX2-ir) in the brains of juvenile and subadult brain but a decline was observed in the adult quail brain (Figure 4.8 A-C), with a consistent distribution pattern across all age groups. In juvenile and subadult brains, low densities of SOX2-ir cells were prevalent in various pallial regions, including the Hyperpallium (H), Mesopallium (M), and Nidopallium (N) and the but the entopallium (E) showed a moderate density (Figure 4.8 F), except for the subadult Mesopallium (M), which shows a high density of SOX2-ir (Figure 4.8 F). In the juvenile brains SOX2-ir cells extend towards the most lateral areas of the pallium at moderate density, whereas the subadult and adult groups had low densities. The distribution pattern in the subadult brains is similar to the juvenile brains; however, in adult brains, the density in pallial areas decreases with age (Table 4.3).

4.3.3 Ventricular zones

In juvenile and subadult brains, the telencephalic ventricles exhibited a high density of SOX2-ir cells along the lining of the lateral ventricles (VZ) and subventricular zones (SVZ) (Figure 4.8 F, A- G) These cells extended throughout the rostrocaudal length but are more concentrated and aggregated in the rostral part. Some of the SOX2-ir cells from the ventricular zones spread into the subpallial areas such as cells accumbens (AC) (Figure 4.8 F).

4.3.4 Hippocampus

The distribution of SOX2-ir cells in the hippocampus is high in the ventral (V) area of both juvenile and subadult quail brains. In the dorsal area of the hippocampus, layer II and its dorsal extent exhibit a moderate density of SOX2-ir cells (Figure 4.8 F, A-C). A moderate density was observed in the area parahippocampalis (APH), dorsolateral

cortical area (CDL), and the piriform cortex (CPI) in the juvenile and subadult quails but there was a decline in the adult brain and ventral area devoid of Sox2-ir cells.

4.3.5 Subpallium

In juvenile and subadult brains, a high to moderate density of SOX2-ir cells were observed in the medial and lateral septum (SM and SL), nucleus posterioris amygdalopalli (POA), globus pallidus (GP) and nucleus accumbens (Ac). A moderate density was spread from the rostral ventricular area into the bed nucleus of stria lateralis (BSTL) fasciculus prosencephali lateralis (FPL), nucleus accumbens (Ac), ventral tegmental area (VTA) and medial striatum (Mst). However, the adult brain showed a decline in all the subpallial structures (Table 4.3, Fig 4.8 E-G, G1-F1).

4.3.6 Diencephalon

In the diencephalon, a high density of SOX2-ir cells were observed in the epithalamic nuclei medial and lateral habenula the in juvenile quails but a moderate to low densities were seen in subadult and adult brains (Table 4.3). SOX2-ir was scattered in the diencephalic core areas in all the experimental groups. The highest density of SOX2-ir cells were localized in the preoptic nucleus (POM), ventromedial hypothalamus (VMN), tuberal nucleus (TU), median eminence (ME), and anterior medial nucleus of the hypothalamus and diencephalic pial surface (Figure 4.9 A-D). In the paraventricular nucleus of the hypothalamus (PVN) (Figure 4.9 G-J). Sox2-ir cells appeared as large cells with cytoplasmic extensions directed towards the anterior commissure, with the highest density observed in the subadult brains (Figure 4.9 E-F). These cells are dispersed and of low density in juvenile and adult birds. In the thalamus, the thalamic nuclei exhibited moderate to low density SOX2-ir cells in nucleus dorsolateralis anterior thalami (DLAl), nucleus dorsomedialis anterior thalami

(DLAm), nucleus rotundus (Rt), and nucleus ovoidalis (OV) and a decline was seen in 12-week-old adult quails (Table 4.3).

4.3.7 Mesencephalon and optic tectum

In juvenile and subadult quail brains, the mesencephalic nuclei exhibited a moderate density in the including the nucleus semilunaris (SLu), oculomotor nucleus (OMN), Edinger–Westphal nucleus (EW), griseum centrale (Gct), nucleus of the basal optic root (nBor), formatio reticularis medialis mesencephali (FLM), nucleus mesencephalicus lateralis, and pars dorsalis (MLd) (Figure 4.10 A). A low density was recorded in the nucleus isthmi, pars magnocellularis (Imc), nucleus isthmi, pars parvocellularis (Ipc), nucleus intercollicular (Ico), and substantia nigra (Table 4.3).

In the optic tectum, the tectal ventricle, stratum opticum (SO) expressed the highest density of SOX2-ir cells in the juvenile and subadult brains. The stratum griseum centrale (SGC), and stratum album centrale (SAC) exhibited a moderate density of SOX2-ir cells. The stratum griseum fibrosum superficiale (SGFS) cells were scattered along its length, with some SOX2-ir cells from the SO spreading into the SGFS layer in juvenile and subadult quail brains (Figure 10.E, F). A low expression of SOX2-ir was found in the stratum griseum (SGP and SGF) layers and a decline was seen in 12-week-old adult quails (Table 4.3)

4.3.8 Cerebellum

The highest density of SOX2-ir cells was localized in the cerebellar pial surface in juvenile and subadult brains. The Purkinje cell layer (PCL) exhibited high density SOX2-ir cells in the juvenile, moderate and low densities in the subadult and adult brain. The molecular (MCL) granule cell layers (GCL) were scattered and showed moderate to low densities in the experimental groups (Figure 11. A- C)

Table 4.3 Summary of qualitative distribution and density of SOX2 immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of SOX2 immunoreactive structures

Brain Region			SOX2-ir cells		
			Juvenile	**Subadult**	**Adult**
Olfactory bulb	OBV		+++	+++	++
Telencephalon	Ventricular zone				
		LV	+++	+++	++
		SVZ	+++	+++	++
		Die VZ	+++	+++	+
		3rd Vent	+++	+++	+
		Tectal Ven	++	++	+
	Pallium	HA	-	-	-
		HI	-	-	-
		HD	++	++	-
		M	+++	++	++
		N	++	++	-
		NC	-	-	-
		E	+	+	-
		A	+	+	-
	Hippocampus	HP	+++	+++	++
		CDL	++	++	+
		CPI	++	++	+
	Subpallium	POA	+++	+++	+
		TSM	+++	+++	+
		GP	++	+++	+
		SL	+++	+++	+++
		SM	+++	+++	+
		FPL	++	+++	+
		VTA	++	+++	++
		AC	+++	+++	++
		BSTL	++	++	-
Diencephalon	Epithalamus	LH	+++	++	+
		MH	+++	++	+
	Hypothalamus	POM	+++	+++	+
		PVN	+	+++	+
		VMN	+++	+++	+
		ME	+++	++	+
		Tu	+++	+++	+
	Thalamus	Rt	+++	+++	+
		OV	++	++	+
		DLA	++	++	+
		DMA	++	++	+

		Glv	++	++	+
Mesencephalon		OMN	++	++	_
		Gct	++	++	+
		EW	++	++	+
		SLu	++	++	+
		FLM	++	++	+
		MLd	++	++	+
		nBor	++	++	+
		IPC	+	+	-
		IMC	+	+	-
		ICO	+	+	-
		Sng	+	+	-
Optic tectum		SO	+++	+++	+
		SGC	+++	+++	+
		SGFS	++	+++	+
		SGF	+	+	-
		SAC	+++	++	+
Rhombencephalon	Cerebellum	Cbi	++	++	+
		MCl	++	+++	++
		Pcl	++	+++	++
		Gcl	+	+	++
	Pons		+++	+++	+
	Medulla		+	+	-

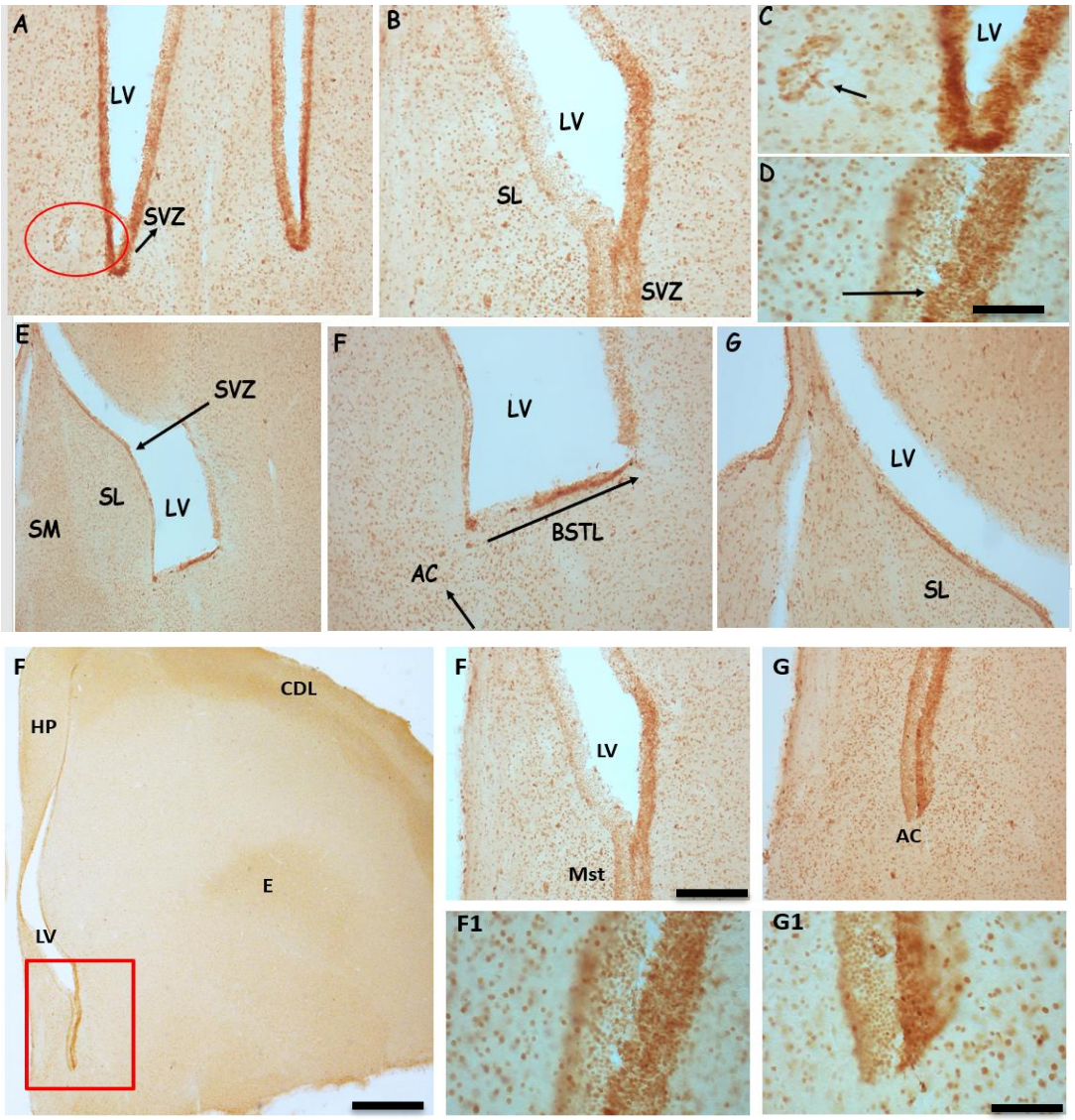


Figure 4.8: Photomicrographs showing the distribution of SOX2 immunoreactive cells in the telencephalic structures. Panoramic view showed the general structures stained by SOX2 **(F).** High power magnification image of SOX2 in the pial surface **(PS) (A-C),** ventricular zones **(VZ)** that are localized at the rostra-caudal area **(A-G).** Hippocampal formation with the V area intensely stained, seen also are the dorsolateral cortical area and piriform cortex **(CDL and CPI)** *(***F, A-C).** In the Subpallium bed nucleus of stria lateralis *(***BSTL),** lateral septum *(***SL***),* **medial septum (SM), cell accumbens (AC)** and medial striatum (**Mst)** have a high density with the lowest seen in the core pallium with the exception of entopallium **(E) (E-G, G1-F1). Scale bar:** F=1mm, **500µm, E-G, F1, G1, H =200µm**

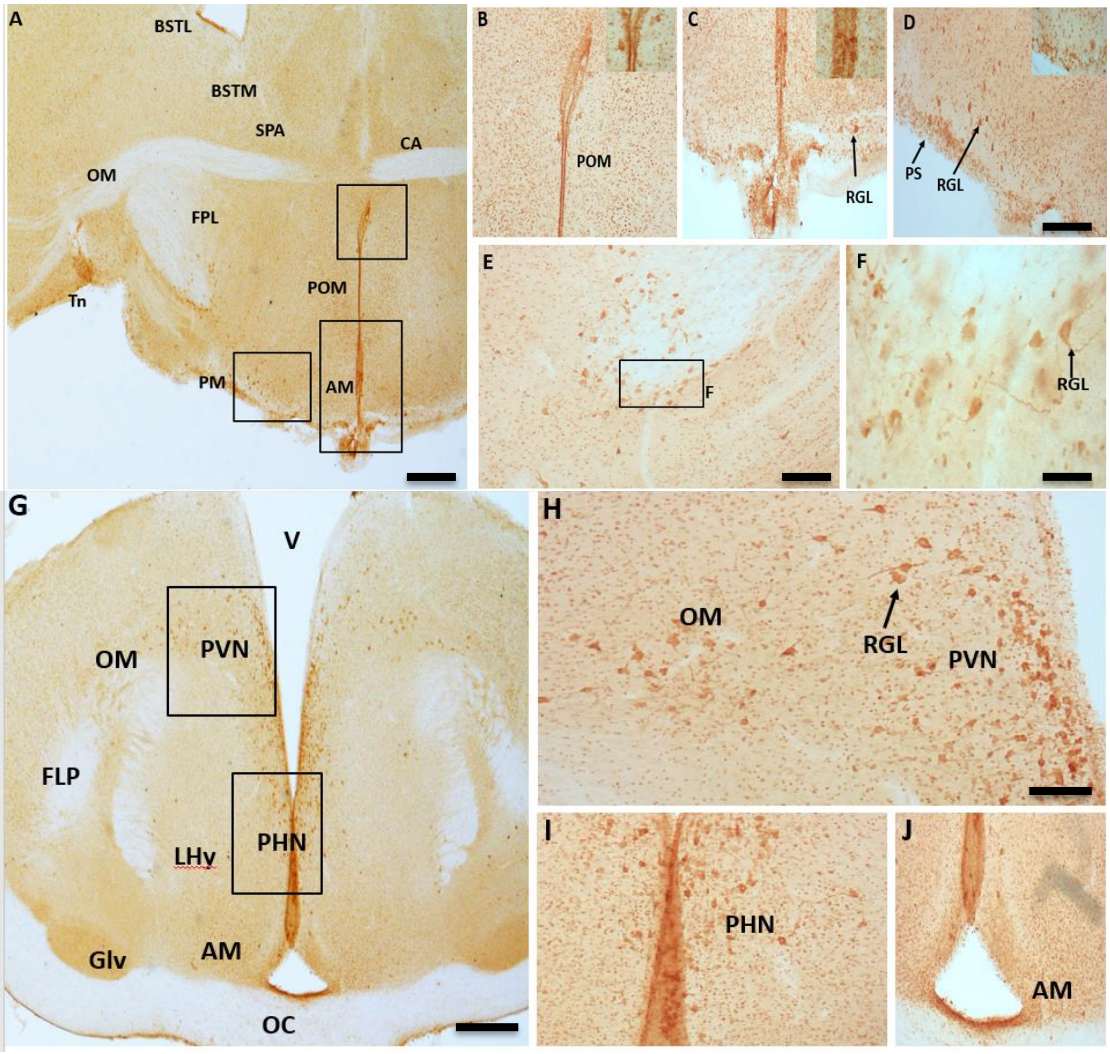


Figure 4.9: Photomicrographs showing the distribution of SOX2 immunoreactive cells in the diencephalon**.** The coronal sections from the rostral to the ventral diencephalon. **A:** panoramic view of the diencephalon showing the hypothalamus through the preoptic nucleus medialis **(POM)** pial surface **(PS)**, **B, D, F** while **C, E, (G and H)** are the corresponding higher power magnification. (**H, I and J)** at the lower magnification showed how the cells from the PVN are migrating towards the OM and have unipolar or bipolar short processes. The SOX2 immunoreactive cells are lining the ventricular wall (**A, H). Scale bar: A, G=** 1mm, **B, C, D, J=**200µm, **E, H, I** = 500µm, **F**= 50µm

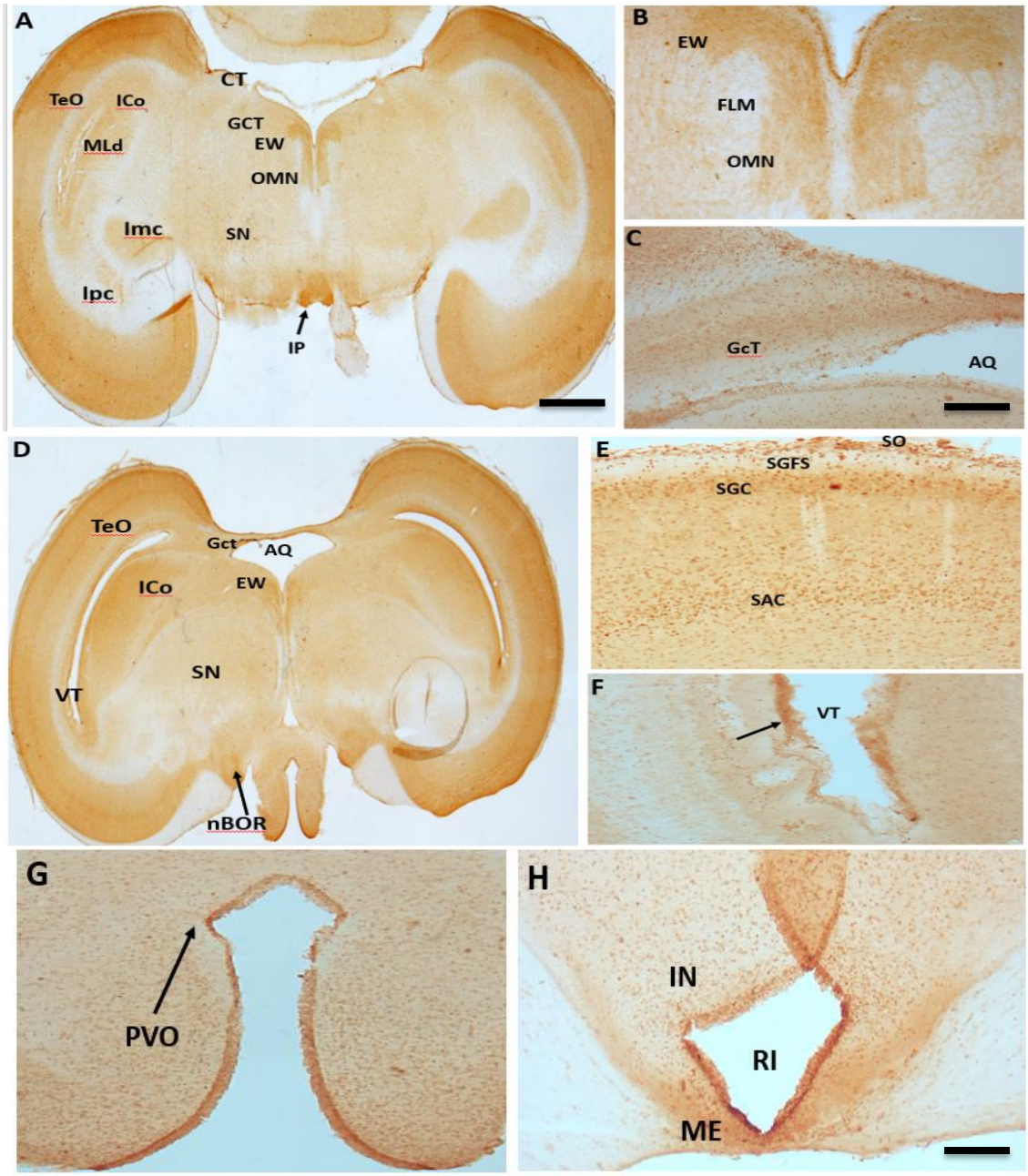


Figure 4.10: Photomicrographs showing the distribution of Sox2 immunoreactive cells in the metencephalon and optic tectum**. A, D:** panoramic view of midbrain through the **EW, OMN, GcT, IMC, IPC, MLD** showed a moderate density A, D and B, C, G, H corresponds to high power magnification. Optic tectum layers **SO, SGC,** ***SAC*** and tectal ventricle **VT** revealed a high density SOX2-ir cells E and F. Scale bar: A, D=1mm, B,C, E =500µm, F, G, H, =100µm.

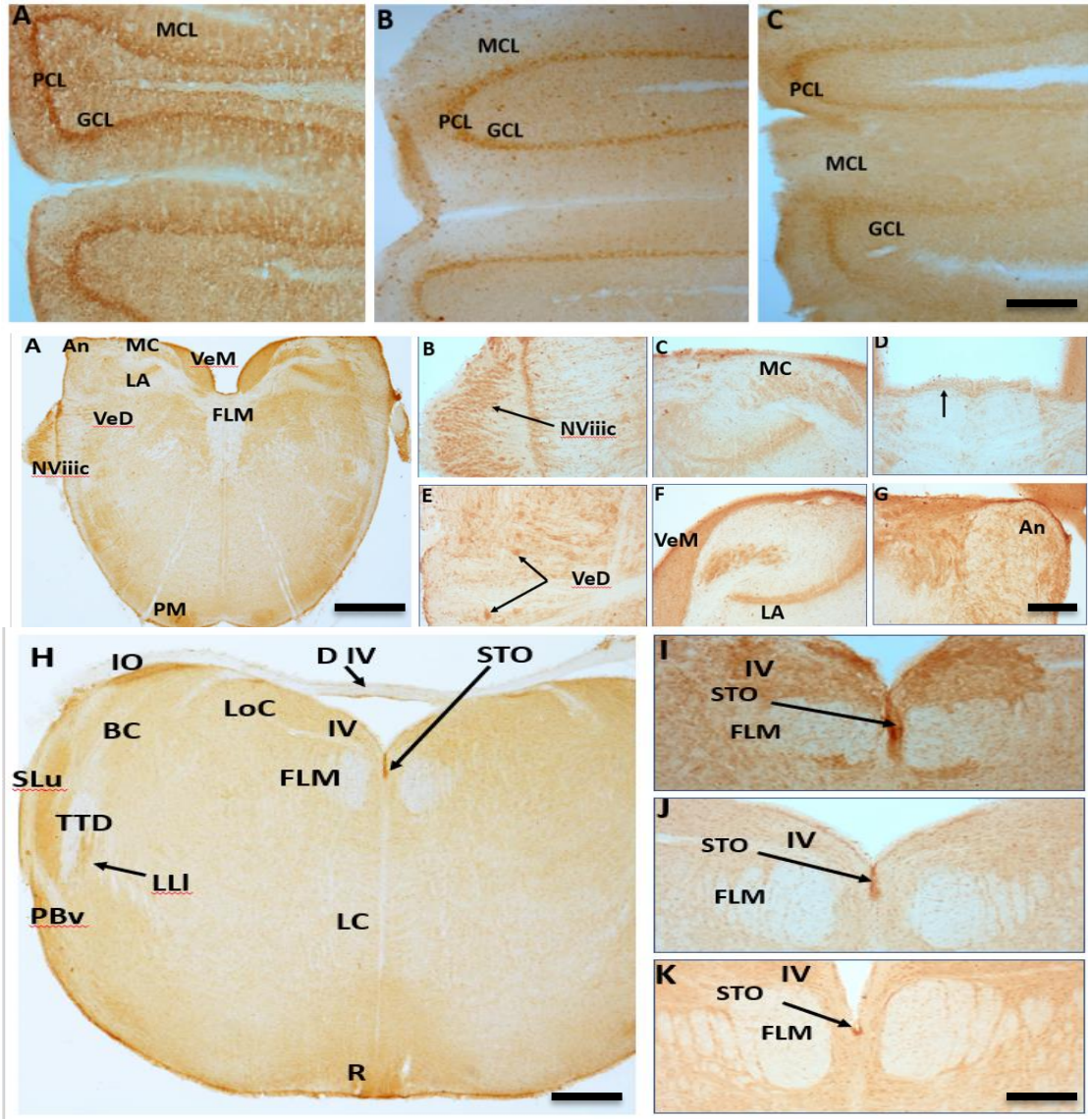


Figure 4.11: Photomicrographs showing the distribution of Sox2 immunoreactive cells in the cerebellum and brainstem**.** SOX2-ir cells are exhibited at a high density in the **PCL** and **oMCL** in the juvenile brain **A,** moderate density in the subadult **B** and the lowest observed in the adult quail **C.** SOX2-ir cells are dispersed at the **GCL** and **MCL**. Panoramic view showing the distribution of immunopositive percussor cells in the brainstem not limited to **SLu, R, VeM IO, PM, and, PBv: A, H.** High power magnification revealed a vigorous staining intensity in the **MCC, VeD, NVIIIc, AN, LA**: B, C, D, E, F. **NIV, STO, FLM**: I, J, and K. **VeD:** B showed a moderate density, large SOX2-ir cells with a bipolar process (radial glial-like). Moderate density expressed in the FLM, LLi, LoC, LC in the juvenile and subadult quail brain, but weakly stained in the adult brain. Scale bar: A, H = 1mm, =500µm, B, C, D, E, G, =500µm, cerebellum A, B, C, and brainstem F, I, J, K=100µm,

4.4 Distribution and characterization cell proliferation identified by PCNA immunoreactive cells in the quail brain

4.4.1 Olfactory bulb

The olfactory ventricular zone (VZ) of the juvenile brain exhibited a high density of cells immunoreactive to proliferating cell nuclear antigen (PCNA-ir), with the greatest concentrations located at the outer edges of the ventricular zone. These cells extend from the ventricular wall towards the hyperpallium densocellulare (HD). A similar distribution pattern was observed in both the subadult and adult brains, although the adult group demonstrated the lowest density of these cells (Figure 4.12A-F).

4.4.2 Ventricular zone

The walls of the lateral ventricle showed a high density of PCNA-ir cells throughout the rostroventral regions, with notable variations across different age groups. In juvenile brains, the highest density of PCNA-ir cells was identified in the ventral areas of the ventricular zone, extending dorsally towards the lateral striatum (Lst), beneath the nucleus accumbens (AC), and the bed nucleus of the stria terminalis lateralis (BSTL). Occasionally, cell clusters and aggregations were observed along the ventricular axis, with some migrating towards the mesopallium, although these were absent in adult brains. Additionally, some PCNA-ir cells were dispersed from the ventricular zone (VZ) wall to the subventricular zone (SVZ). The sub-adult and adult groups exhibited similar distribution patterns. The third, fourth, and tectal ventricles contain moderate densities of PCNA-ir cells (Figure 4.13A-I).

4.4.3 Pallium

In the hippocampi of juvenile quails (HP), PCNA-ir displayed varying densities across different regions. The V area/triangular area and Vm exhibited moderate density, whereas the APH and CDL contained fewer scattered moderate cells. The Vl area showed low density. PCNA-ir expression decreased in subadults and was absent in 12-week-old adults. The distribution of PCNA-ir cells was uneven in the pallial subdivisions. Moderate density was observed in the mesopallium (M), hyperpallium densocellulare (HD), arcopallium (A), and lamina arcopallialis dorsalis (LAD). A low density was noted in the entopallium (E) and field area 2 (L2), with other pallium areas lacking PCNA-ir cells. In subadults, PCNA-ir was absent in the entopallium, low in the mesopallium, and completely absent in the adult quail brains. The subpallium exhibited moderate density in the nucleus accumbens (Ac), medial striatum, medial septum, BSTL, Posterior Nucleus of the pallial amygdala (POA), and taenial amygdala (Tna) and low density in the lateral striatum. Areas, such as the globus pallidus (GP), nidopallium (NCL), hyperpallium apicale (HA), and hyperpallium intercalatum (HI) lack PCNA-ir cells. Subadults showed a similar pattern of PCNA-ir immunoreactive cells that were absent in adults (Figure 4.14A-C).

4.4.4 Diencephalon

In subadult quail, the lateral habenula (LH) exhibited a moderate density of PCNA-immunoreactive (PCNA-ir) cells, whereas the medial habenula (MH) demonstrated a low density. Conversely, both the lateral and medial habenulae in juvenile and adult quail brains displayed low cell density (Figure 4.15A-C). In juvenile and subadult quails, the nucleus rotundus (Rt) exhibited a high density of PCNA immunoreactivity, whereas the adult brain showed lower levels. The juvenile nucleus ovoidalis (OV), nucleus dorsomedial anterior thalami (DMA), and nucleus dorsolateral anterior

thalami (DLA) displayed moderate density, whereas the geniculatus lateralis pars ventralis (Glv) had low density. Although the distribution pattern remained consistent, the subadult group demonstrated the highest density, whereas the 12-week adult brain had the lowest density, with Glv being absent in adults. The thalamus showed intense PCNA immunoreactivity in the Rt; moderate PCNA-ir cells in the DMA, DLA, and Glv; and few PCNA-ir cells in the OV. In the adult brain, PCNA-ir cells in the thalamus were generally low in density, except for Rt, which maintained moderate intensity, but with fewer PCNA cells compared to juveniles (Figure 4.15D-F).

The preoptic nucleus (POM) exhibited the highest density of proliferating cell nuclear antigen (PCNA) in the hypothalamus of the juvenile and subadult specimens. Moderate PCNA density was observed in the nucleus tuberis (Tu), nucleus paraventricularis (PVN), and nucleus ventromedialis hypothalamic (VMN). The anterior commissure also showed moderate density with occasional high-density areas. In the caudal region adjacent to the median eminence (ME), The optic chiasma displayed a few scattered cells with a moderate density in the caudal region adjacent to the ME. Adult brains demonstrated a similar expression pattern, although the density decreased with age (Figure 4.15G-L).

4.4.5 Mesencephalon and Optic Tectum

In the midbrains of juvenile and subadult specimens, several structures showed a moderate density of PCNA-immunoreactive (PCNA-ir) cells. These structures included the Gct, Ru, tractus occipitomesencephalus, commisura tectalis (CT), nucleus isthmi, pars magnocellularis (Imc), nucleus isthmi, pars parvocellularis (Ipc), nucleus intercollicularis (Ico), and nucleus of the basal optic root (nBOR).

The highest density of PCNA-ir cells was observed in the Edinger Westphal (EW), oculomotor nerve nuclei (OMN), subcommisural organ (SCO), nucleus spiriformis medialis and lateralis (SPm and SPl), mesencephaly nervi trigemini (NVm), and their adjacent structures. Certain regions, such as the tractus isthmus-opticus (Tio), nucleus mesencephalicus lateralis, pars dorsalis (MLd), and substantia nigra pars compacta (SNc), exhibited low counts of PCNA-positive cells. However, the formatio reticularis medialis mesencephali (FLM) was devoid of these cells across all experimental groups. Within the optic tectum, a low density of PCNA-ir was detected in the stratum albumen centrale (SAC), stratum griseum centrale (SGC), and the ventricular mesencephala (VT). The remaining layers of the optic tectum and the adult brain were devoid of PCNA-ir cells in this region (Figure 4.16 A-K).

4.4.6 Cerebellum

The outer molecular layer and lingula (L) of the juveniles and subadults exhibited a high density of PCNA-ir cells. The Purkinje cell layer (PcL), nucleus cerebella internus (CBI), and nucleus cerebella lateralis (CBL) display densities ranging from high to moderate density (Figure 4.17 J-L). In contrast, the granule cell layer (GcL) and internal molecular layer (IML) contained fewer PCNA-ir cells at low density. These cells were absent in the adult brain (Figure 4.17 A-L).

4.4.7 Brain Stem

High PCNA expression was observed in several nuclei of pons and medulla oblongata. In subadults, numerous PCNA-immunoreactive cells were detected in various brain structures, including the nucleus magnocellularis cochlearis (MCC), nucleus laminaris (La), nervus octavus, pars cochlearis (NVIIIc), nucleus nervi abducentis (nVI), nix, nucleus reticularis paramedianus (RPaM), nucleus et tractus descendens nervi

trigemini (TDV), nucleus pontis medialis (PM), nucleus reticularis pontis caudalis (RP), medial and dorsal vestibular nucleus (Vem and Ved), and nucleus reticularis pontine caudalis, pars gigantocellularis (RPgc). Additionally, a large cell adjacent to the median raphe (R) exhibited a high density of PCNA-ir cells. Moderate PCNA-ir cells were also found in structures, such as the locus coeruleus, nucleus isthmus opticus, nucleus olivaris superior, and nucleus sensorius principalis nervi trigemini (Loc, Io, os, and nPrV). These structures are largely absent in the brains of juvenile and adult quails (Figure 4.18 A-T).

Table 4.4 Summary of qualitative distribution and density of PCNA immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of PCNA immunoreactive structures

Brain Regions		PCNA-ir cells			
			Juvenile	Subadult	Adult
Olfactory bulb	OBV		+++	+++	+
Telencephalon	LV		+++	+++	++
	Pallium	PMt	+++	+++	+
		HA	−	−	−
		HI	++	+	−
		HD	+	+	−
		M	+	+	−
		N	+	+	−
		NC	−	−	−
		E	++	+	−
		A	+	++	−
	Hippocampus	HP	+++	+++	+
		APH	+++	+++	++
		CDL	+++	+++	+
		CPI	+++	+++	+
	Subpallium	Mst	++	++	+
		Lst	++	++	+
		GP	−	−	−
		SL	++	++	_
		SM	_	+	_
		BST M	_	_	_
		BSTL	+	++	_
Diencephalon	Hypothalamus	POM	+++	+++	+
		PVN	+++	+++	+
		VMN	+++	+++	+
		Tu	+++	+++	+
	Thalamus	Rt	+++	+++	+
		OV	++	+++	+
		DLA	++	++	+
		DMA	++	++	+
		Glv	+++	++	+
Mesencephalon		Gct	+++	+	−
		OMN	+++	+++	+
		RMC	++	++	+
		Spm	++	+++	+
		Spl	++	+++	+

		CP	++	+	_
		SCO	+++	+++	++
		IF	_	++	_
		1N	_	++	_
		IH	_	++	_
		RU	+++	+++	++
		NI	+++	+++	+
		NIII	++	++	+
		Nvm	+++	+++	++
Optic tectum			++	++	_
Rhombencephalon	cerebellum	Cbi	++	++	−
		Mcl	+	+	−
		oMcl	+++	+++	++
		PCL	++	+++	+
		Gcl	+	+	−
	Pons		+++	+++	+
	Medulla		+++	+++	+
	Cranial nerve		+++	+++	+
	Brain stem		+++	+++	+

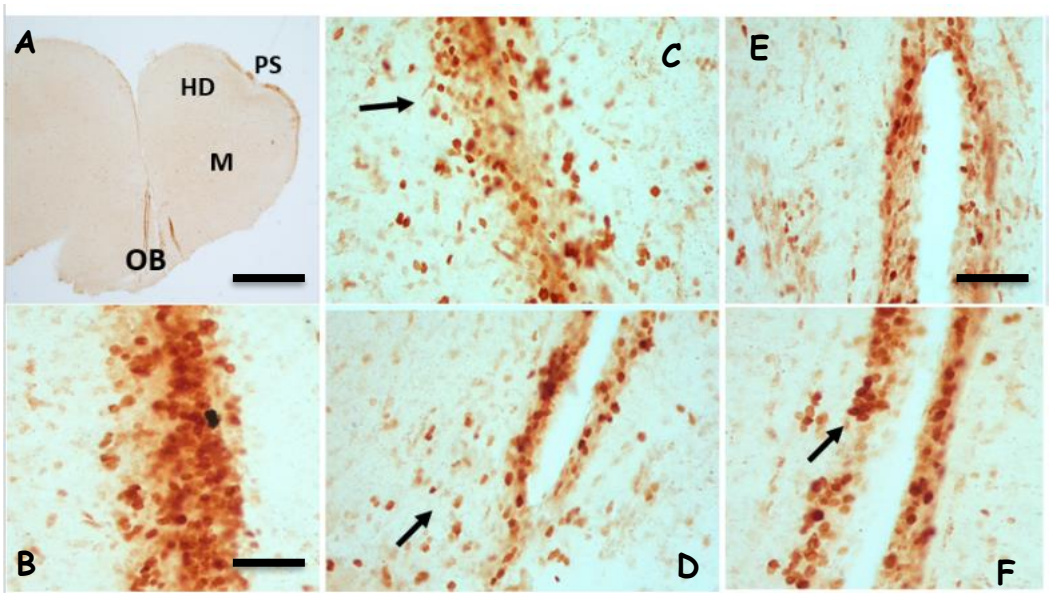


Figure 4.12: Photomicrographs showing the distribution of proliferating cell nuclear antigen (PCNA) immunoreactive cells (PCNA-ir) in the olfactory bulb ventricle (OBV) with a high density of immunopositive cells along the olfactory ventricular wall. (A): panoramic view of OBV in the juvenile brain (B, C, D and F) showed the corresponding higher power magnification indicating cell aggregation, (C and D) has PCNA-ir spreading. The scale bar: A= 1mm, 500µm, B, C, D, E and F=500µm.

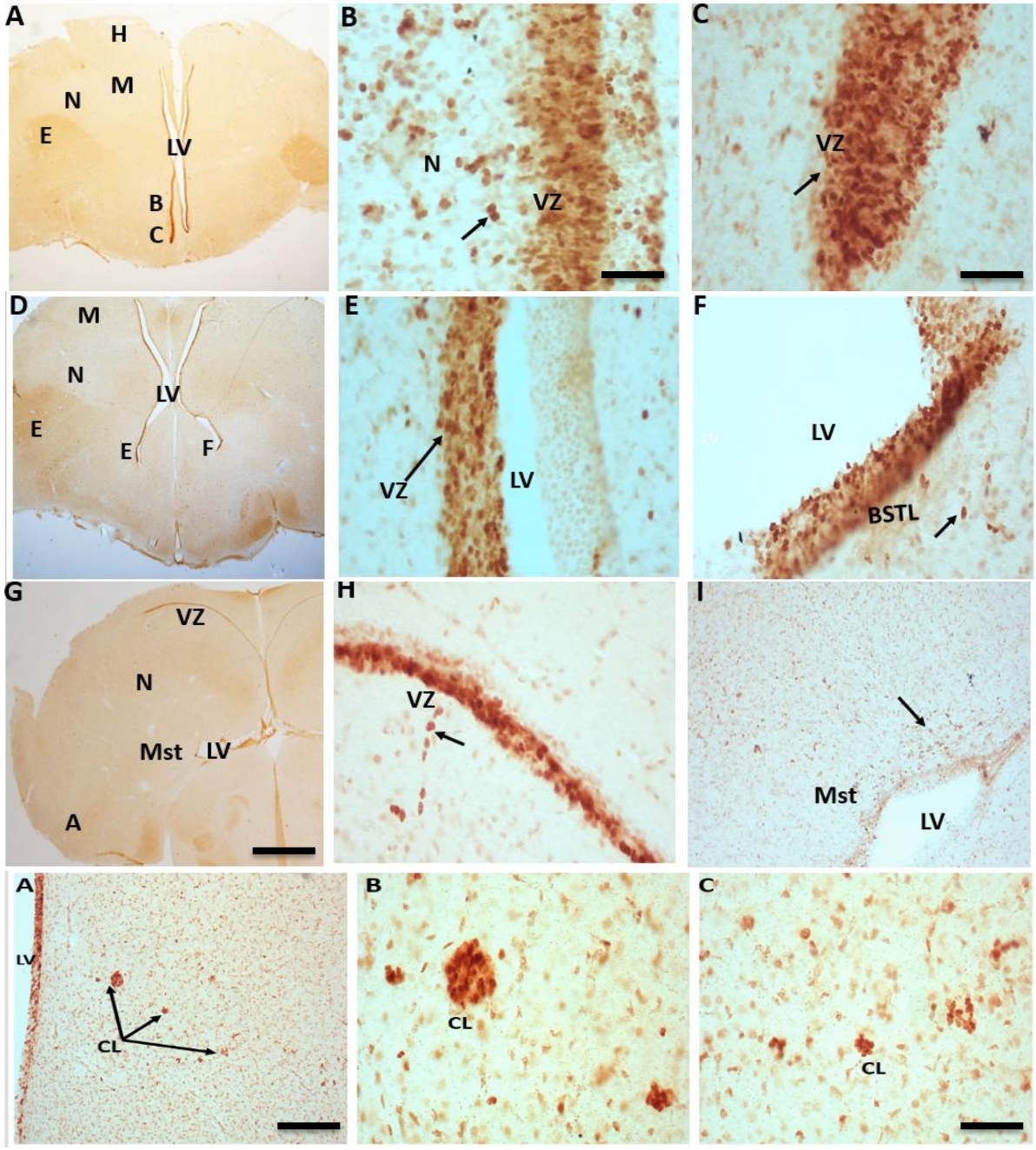


Figure 4.13: Photomicrographs of the distribution of proliferating cell nuclear antigen (PCNA) immunoreactive cells (PCNA-ir) sections through the ventricular zones (VZ) of the lateral ventricle in the juvenile quail brain**. (A, D, G)** panoramic view of the rostral, middle and caudal telencephalon showing the ventricular zone (VZ). **(B, C, E, F and H)** are the corresponding higher power magnification revealing the high density of cell proliferating cells (hot spots). (**A, D, G, I)** revealed pallial and subpallial structures, while (**J, K, L)** show the presence of choroid plexuses**, (B, H and I)** showed scattered cells from the VZ **(M, N, O)** show cell aggregation and clusters from the VZ "hot spots" migrating towards the pallial area. The scale bar: A, D, G =1mm, A I, =200µm , C, E, F H =500µm, B,C=50µm.

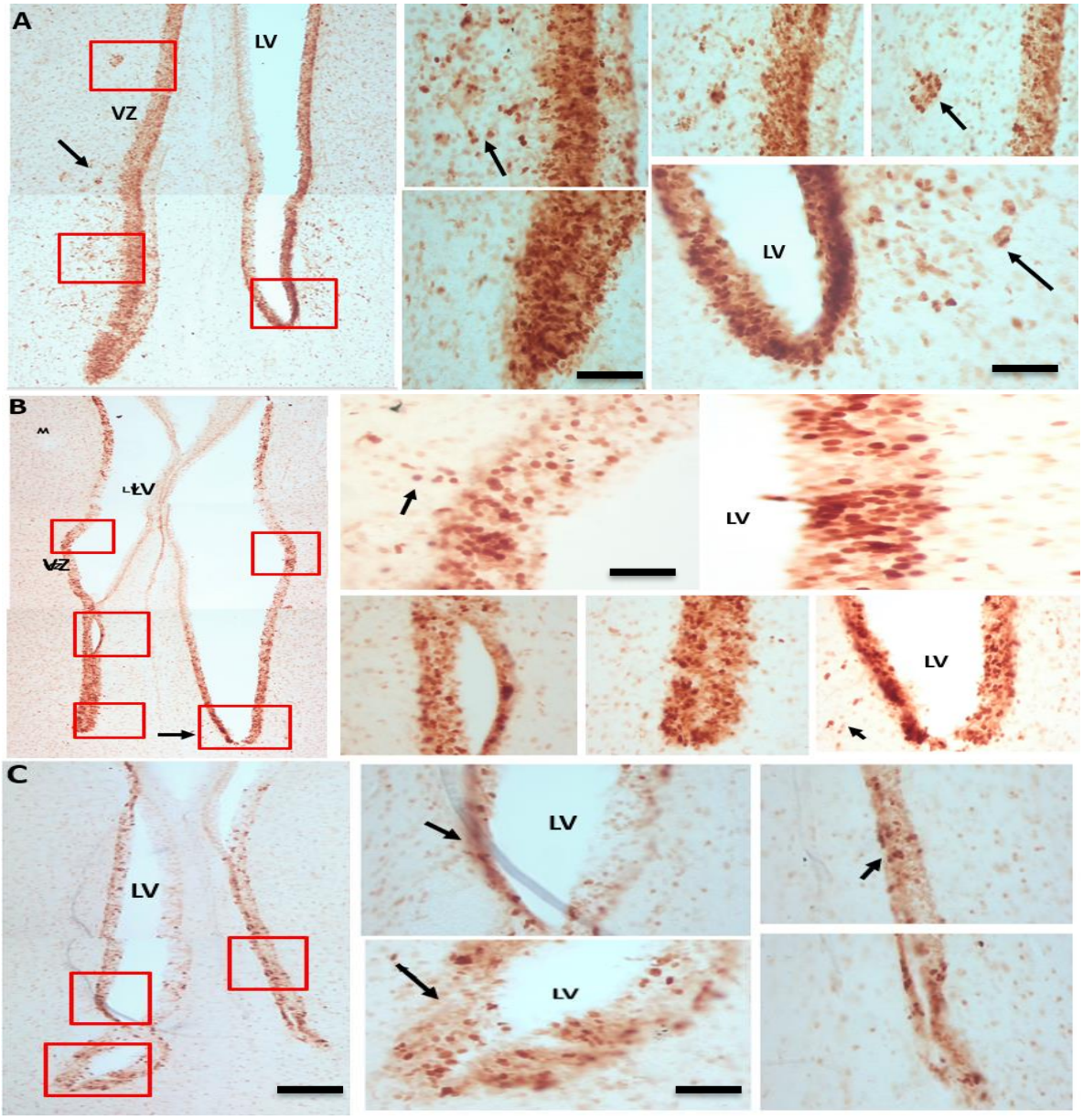


Figure 4.14: Photomicrographs of the juvenile ventricular zone (VZ) of the lateral ventricle (**A)** which exhibit a high density of (PCNA-ir) in the juvenile. The highest density is observed in the ventral area of the ventricular wall, and the proliferative cells are scattered adjacent to the VZ. Middle area showed a cell cluster, the proliferative "hot spot". Similar distribution pattern is observed in the subadult **(B)** and adult (**C) VZ,** however, PCNA-ir are heterogenous and more in the ventral and rostral area as to the middle, though lowest density in the adult brain. The scale bar: A, B, C, =100µm, A, B, C, =50µm.

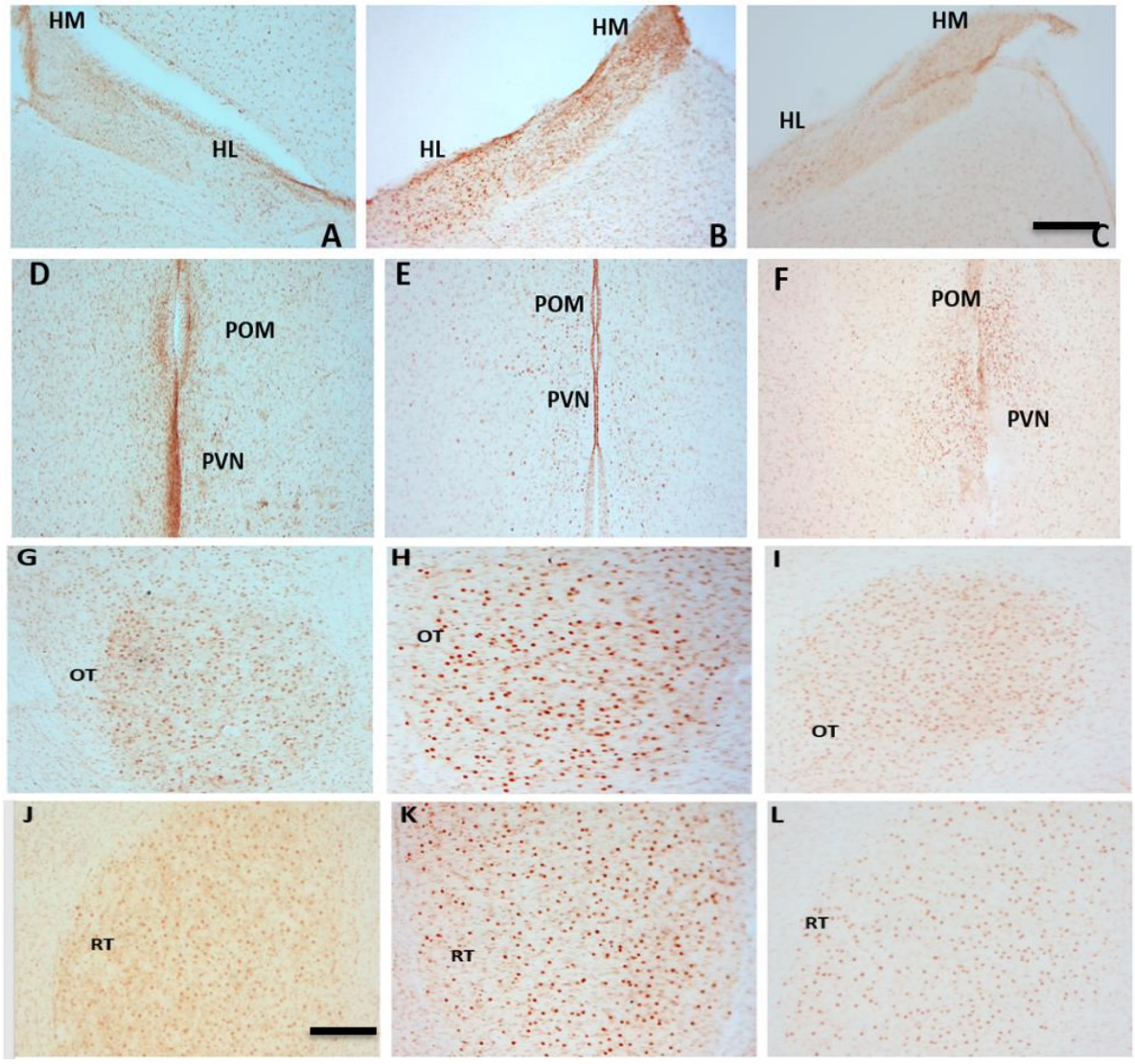


Figure 4.15: Photomicrographs of the distribution of proliferating cell nuclear antigen (PCNA) immunoreactive cells (PCNA-ir) sections through the diencephalon of the juvenile, subadult and adult quail brain at high power magnification**.** Epithalamus shows the distribution in PCNA in HL and HM **(A, B, C).** Hypothalamic nucleus POM, PVN (**D-F**) and thalamic nuclei such as OV and RT **(G-L).** The subadult HL, HM, OV and RT have the highest density compared to the juvenile and adult groups. The scale bar: A- F =100µm, G-L=500µm.

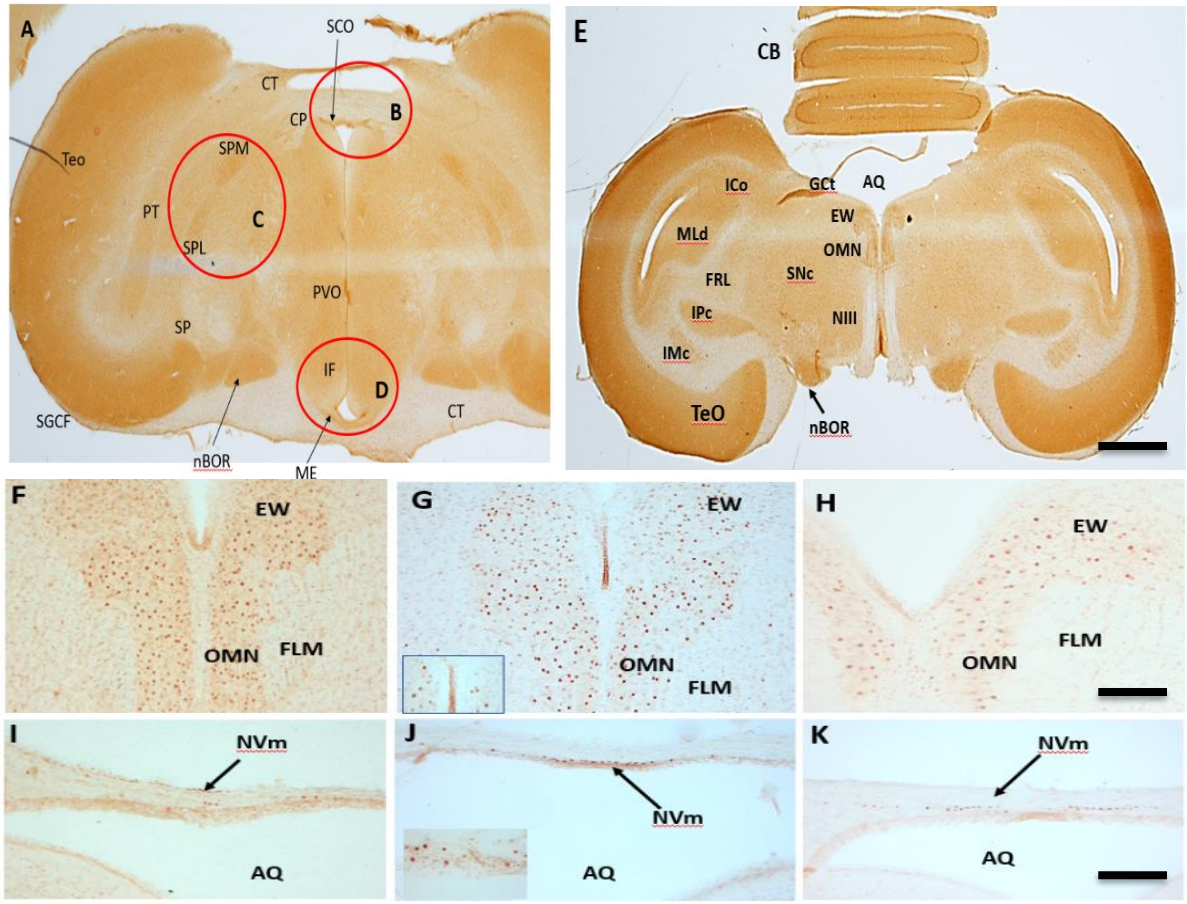


Figure 4.16: Photomicrographs of the distribution of proliferating cell nuclear antigen (PCNA) immunoreactive cells (PCNA-ir) sections through the mesencephalon and optic tectum, panoramic view (**A, E).** Most of the mesencephalic nucleus **SCO, SPl, SPm, OMN, EW, IN IH, IF** and **NVm** revealed in the higher power magnification showed highest density of cell proliferation **(B-K)**. Tectal ventricle **(VT) (L, M, N)** showed a moderate density in the juvenile and subadult group with a very low density in the adult brain. **SAC** layer of the optic tectum **(TeO) (O, P, Q)** has a moderate density in the subadult **(P), SGC** and **SGP** are of low density in both juvenile and subadult groups but totally devoid in all adult tectal layers **(Q)**. The scale bar: A, E, = 1mm, F-K =500µm, the scale bar in the insert (G, J) =50µm.

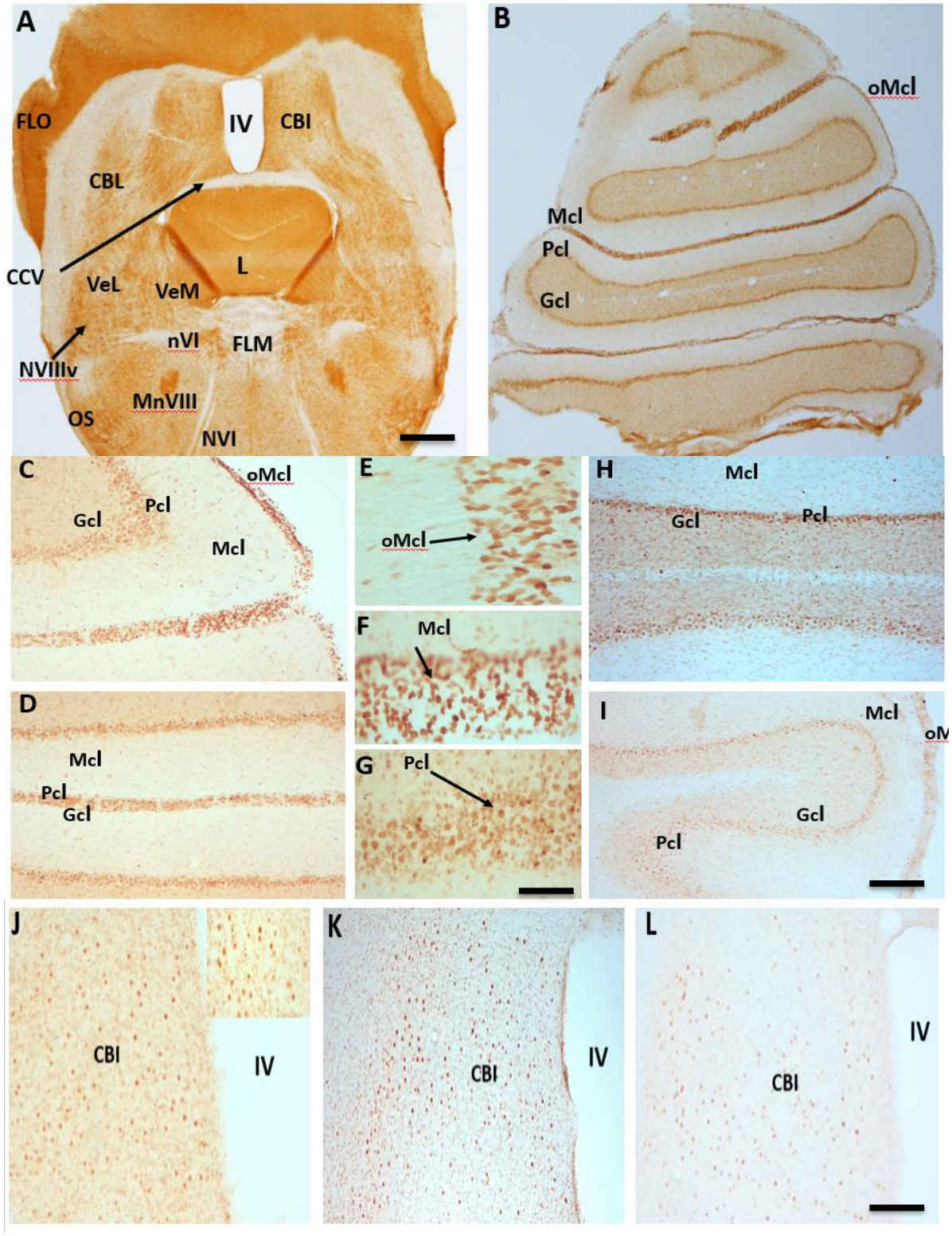
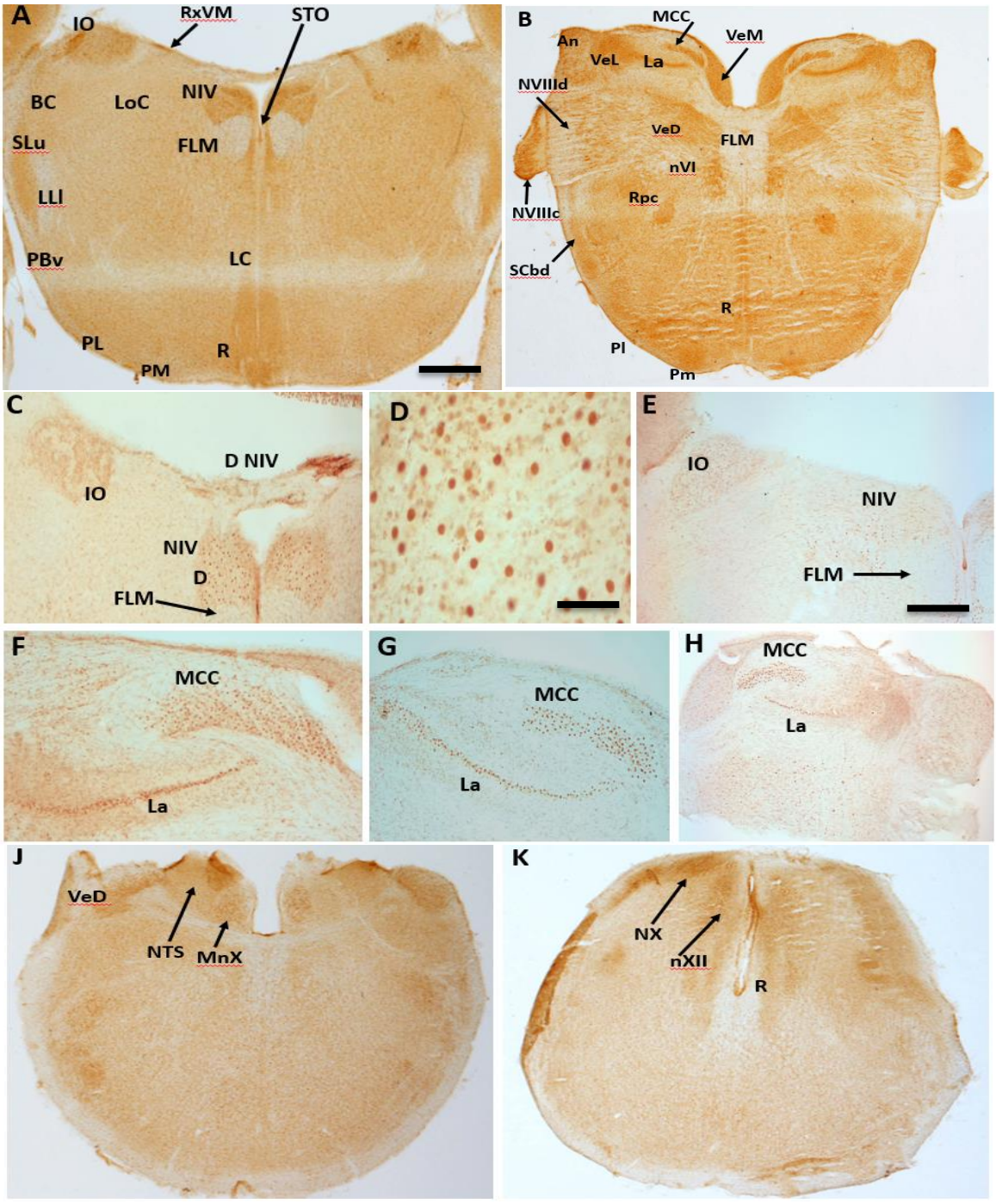

A
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Figure 4.17: Photomicrographs of the distribution of proliferating cell nuclear antigen (PCNA) immunoreactive cells (PCNA-ir) sections through the cerebellum. Panoramic view of the cerebellar layers, cerebellar nuclei and the fourth ventricular recess (**A, B).** High power magnification shows that the juvenile and subadult **OMcl, Pcl**, and **CBI** exhibited the highest density and a few scattered low densities of PCNA-ir are distributed in the Mcl and Gcl (**C, D, H, I).** The higher power magnification of the corresponding area **(E, F, G).** The CBI of the subadult **(K)** has the highest density with a low density in the ependymal lining of the 4^{th} ventricle as compared to the juvenile and adult brain**.** A similar pattern of distribution is seen in the adult quail but there was a decline in the 12 weeks adult brain (**I, L).** The scale bar: A, B = 1mm, E, G, G =50µm, J-L=500µm, C, H, D, 1=200µm.


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NIV
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LLl
PBv
LC
PL
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B
MCC
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VeL
La
VeM
NVIIId
VeD
FLM
nVI
Rpc
NVIIIc
SCbd
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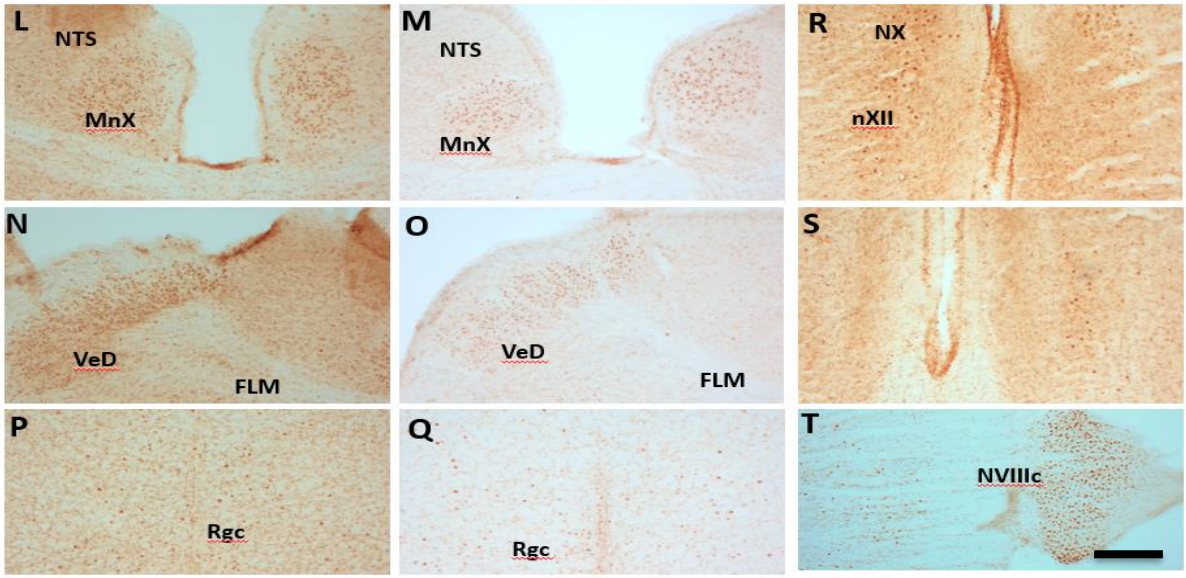


Figure 4.18: Photomicrographs of the distribution of proliferating cell nuclear antigen (PCNA) immunoreactive cells (PCNA-ir) sections through the brain stem with a panoramic view of the basal structures **(A, B**). The basal nuclei in the juvenile nucleus showed a high density of PCNA-ir in the pontine and medullary nuclei such as **IO, D NIV, MCC, La (C, D, F) VeD, MnX, NTS and NX (J, K, L, N, P, R, S)** corresponding structures in the adult are seen in **(E, H, M, O, Q)** while the subadult group is revealed in **(G, T).** The scale bar: A, B, J K =1mm, P, Q =500µm, C,E,F,G,H,L-S, T =200µm, D=50µm.

4.5 Distribution and characterization of astrocytes identified by GFAP-ir in the quail brain.

4.5.1 Olfactory bulb

GFAP-immunoreactive cells were observed to exhibit a higher density in the olfactory bulb of both juvenile and subadult quails. These cells presented a star-shaped morphology, characterized by rounded cell bodies and short to semi-long processes. However, a lower density of these cells was observed in the brains of adult quails.

4.5.2 Pallium, Pial Surface, and Sub pallium

GFAP-immunoreactive cells were absent in the HP region of juvenile quails, present at moderate density in subadults, and present at high density in the adult HP region. In the HA region, the density was observed to be moderate in juvenile quails but high in subadult and adult quails. In the E region, GFAP immunoreactivity was moderately dense in juvenile and subadult brains but absent in the adult brain. The pial surface of the quail brain exhibited a high density of GFAP-immunoreactive cells across all age groups (juvenile, subadult, and adult) (Figure 4.19 A-C & 20 A-C). Blood vessels large and small were GFAP-immunoreactive in all the groups but declined in the adults (Figure 4.25 A-C).

4.5.3 Diencephalon

In the epithalamus, the medial habenula and lateral habenula exhibited a dense distribution and population of GFAP-immunoreactive (Gfap-ir) cells across all age groups (juvenile, subadult, and adult).The Thalamic nuclei (DMA and Glv) exhibited a high density of GFAP immunoreactivity in both juveniles and subadults; in these regions, the cells presented a stellate morphology with moderate branching processes, which was not observed in adult quails. The hypothalamus regions (POM and PVN)

demonstrated a high density of GFAP-immunoreactive cells in both juveniles and subadults, which was not observed in adult quail (Fig. 4.21 A-C, 4.22 A-C).

4.5.4 Mesencephalon and optic tectum

The mesencephalic region Gct exhibited a high density of GFAP-immunoreactive cells in both juveniles and subadults but was absent in adult quails. The optic tectum region SO demonstrated a high density of GFAP-immunoreactive cells across all age groups of quails. However, in the regions (SGFS, SGF, and SAC), GFAP-immunoreactive cells were observed only in juveniles and subadults (Table 4-5). The third ventricle and SCO exhibited a dense distribution and population of GFAP-immunoreactive cells across all age groups (juvenile, subadult, and adult). The third ventricle region demonstrated highly populated GFAP-immunoreactive cells, ranging in morphology from short ramified to moderately shaped processes. Conversely, IF, CT, and CP were observed to have densely populated GFAP-immunoreactive cells in both the juvenile and subadult brain, which were absent in the adult brain. The Edinger-Westphal nucleus (EW) region exhibited a moderate density of GFAP-immunoreactive cells in the brains of both juvenile and subadult quails; however, these cells were absent in the EW region of adult quails (Table 4-5). The cellular structure of these cells was observed to possess a short-ramified process (Figure 4.23 A-C, 4.24A-C).

4.5.5 Rhombencephalon (cerebellum, pons, and medulla)

The cerebellum molecular (Ml) and Purkinje cell layer (Pcl) exhibited a high density of GFAP-immunoreactive cells in the juvenile, which persisted exclusively in the Pcl of the adult quail's brain. In contrast, only the adult granular cell layer (Gcl) demonstrated a moderate distribution of GFAP-immunoreactive cells. The cell morphology presented a fusiform structure with elongated branching processes, with

each process connecting to adjacent processes, which was concentric across the brain regions and traversed from one layer of the cerebellar cortex to another (from the granular layer to the Purkinje cell layer). These cells were not observed in the cerebellar white matter layer. In the pons, a high density of GFAP-immunoreactive cells was observed in both juveniles and subadults (Fig. 4.25 A-C).

The fourth ventricle, tectal ventricle, fasciculus longitudinalis medialis (FLM), nucleus isthmi pars parvo-cellularis (IPC), and nucleus isthmi pars magno-cellularis (IMC) all demonstrated a variation ranging from moderate to densely populated GFAP-immunoreactive cells in the brains of Japanese quails of all age groups (juveniles, subadults, and adults) (Table 4-5).

Table 4.5 Summary of qualitative distribution and density of GFAP immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of GFAP immunoreactive structures

Brain Region			GFAP-ir cells		
			Juvenile	Subadult	Adult
Olfactory Bulb	OBV		+++	+++	+
Telencephalon	LV		—	—	—
	Pallium	HP	+++	+++	+
		APH	+++	+++	+
		CDL	+++	+++	+
		CPI	+++	+++	++
		HA	+	++	++
		HI	—	—	—
		HD	—	—	—
		M	—	—	—
		N	—	—	—
		NC	—	—	—
		E	+	+	—
	Pial Surface	PS	+++	+++	+++
		A			
	Sub pallium	Mst			
		Lst			
		GP	+++	+++	+
		SL	+	+	—
		TN			
		SM	+	+	—
		BSTM			
		BSTL			
Diencephalon	Hypothalamus	POM	+++	+++	—
		PVN	+++	+++	—
		VMN			
	Thalamus	Rt			
		OV			
		DLA			
		DMA	++	++	—
		Glv	+	+	—
Mesencephalon		Gct	+++	++	—
		OMN			
		RMC			
Optic Tectum		SO	+++	+++	++
		SGFS	++	++	—
		SGF	++	++	—
		SAC	+++	++	—
Rhombencephalon	Cerebellum	Cbi			
		MI	+++	+++	—
		Pcl	+++	+++	++
		Gcl	—	—	+
	Pons	Nville	+++	+++	—
	Medulla	LPO	+	+	—

	Wall of VT	VZ v	++	++	—
		VZ d	—	—	
		FPL	+	+++	+
		PA	+	+	+++
		AC	+	+	—
	Third Ventricle		+++	+++	+
	Medial habenula		+++	+++	++
	Lateral habenula		+++	+++	+
		PVO	++	++	+
		IF	+++	+++	—
		CT	+++	+++	—
		CPI	+++	+++	—
		SCO	+++	+++	++
	Fourth Ventricle		++	+++	+
	Tectal Ventricle		++	+++	+++
		EW	++	++	—
		NIII	++	++	++
		FLM	++	++	
		IPC	++	++	+
		IMC	++	++	
		Tio	++	+++	—
		Io	++	+++	—
		Tic	+	++	—
		Rpgc	+	+	—
		Ru	+	+	—
		Fu	++	++	—
		VEM	++	++	—
		RP	+	+	—
		mcc	++	++	—
		Tov	+	+	—
		CA	—	—	++
		OC	++	++	+

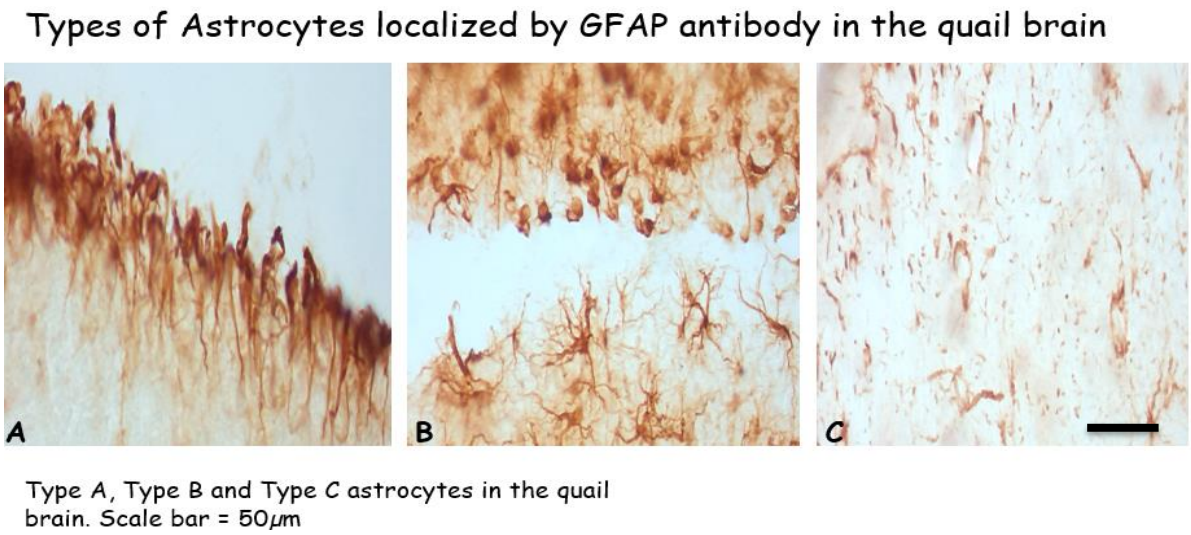


Figure 4.19: Photomicrograph of GFAP immunopositive cells in the juvenile, subadult and adult quail brain – Three types of astrocytes were observed. **Type A** has long processes which are perpendicularly found in the pial surface (**PS)** and hippocampus **(Hp). Type B:** that has a soma with multi-polar processes seen in large ventricles (**LV)** and grey matter across the brain. **Type C:** Soma is empty with little or no processes, found in the white matter. **(A – C):** different types of astrocytes. Scale bar: **C : 50µm,** applies to all

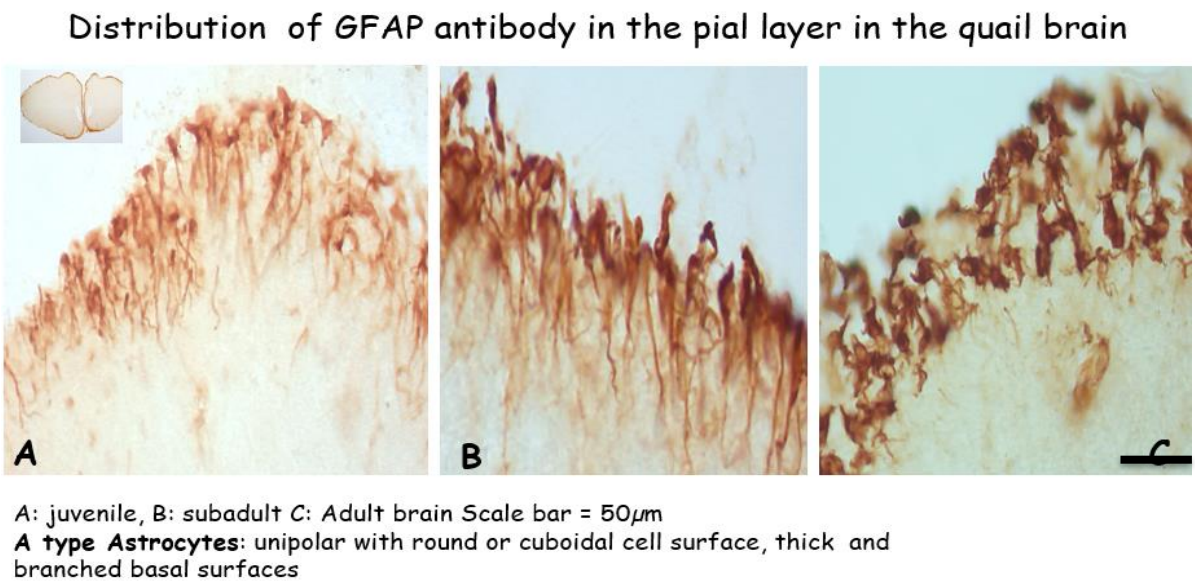


Figure 4.20: Photomicrograph of GFAP Type A immunopositive cells in the pial surface of juvenile **(A)**, subadult **(B)** and adult **(C)** quail brain. They are darkly stained body and elongated processes. GFAP positive cells are absent in the core pallial regions. **Scale bar : C= 50µm**, applies to all

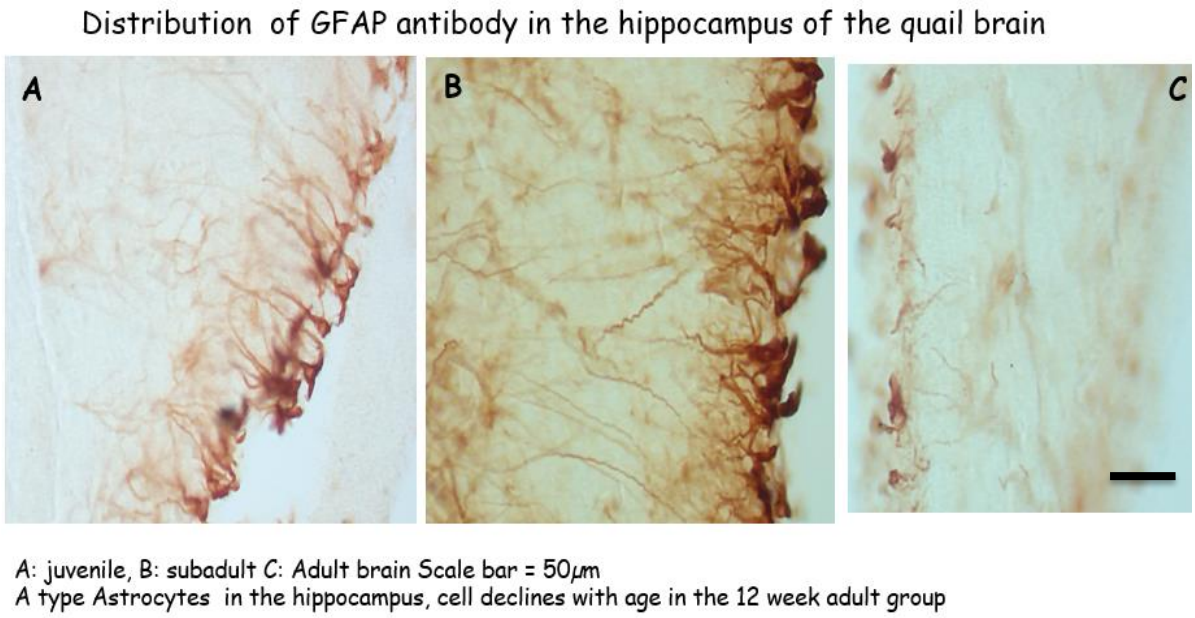


Figure 4.21: Type A GFAP immunopositive cells observed in the hippocampus of juveniles, subadult and adult quail brain. The juvenile **(A)** has a moderate density while the subadult **(B)** exhibited the highest density and long processes running perpendicular from the edge to the core hippocampus area. The decline is well pronounced in the adult **(C)** brain, almost lost the processes and low in density. Scale bar **C : 50µm**, applies to all

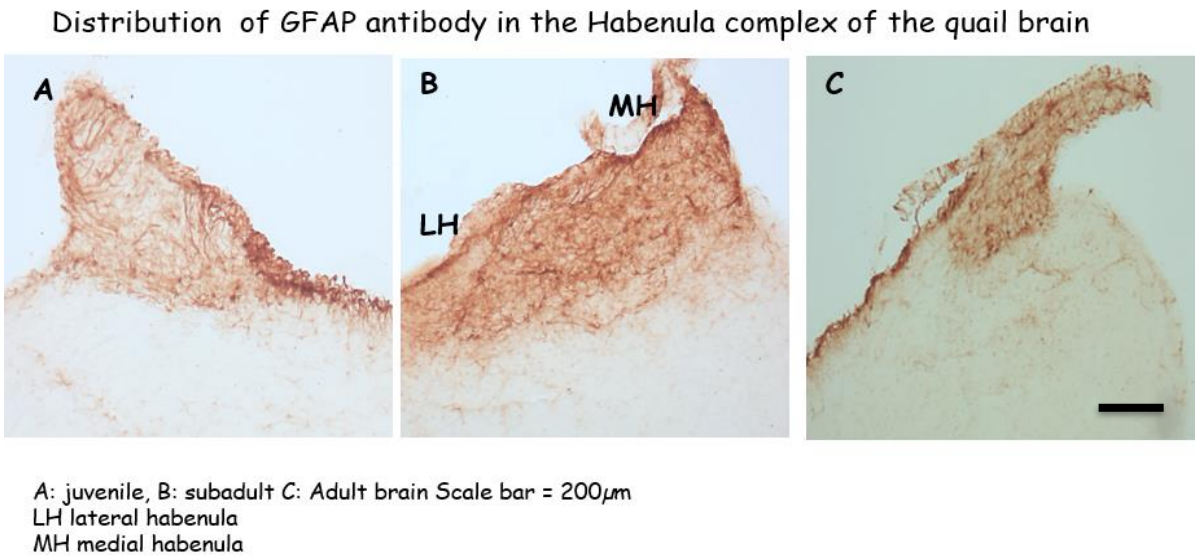


Figure 4.22: Photomicrograph of GFAP Type A immunopositive cells in the epithalamus, both the medal habenula **(MH)** and lateral habenula **(LH)** of the juvenile, subadult and adult brain. They are darkly stained body and processes. The juvenile **(A)** and subadult **(B)** brain showed a high density but much in the subadult group. There was a decline in the adult **(C)** brain. Most of the epithalamic nuclei are devoid of GFAP immunoreactive cells. Scale bar : **C : 50µm**, applies to all

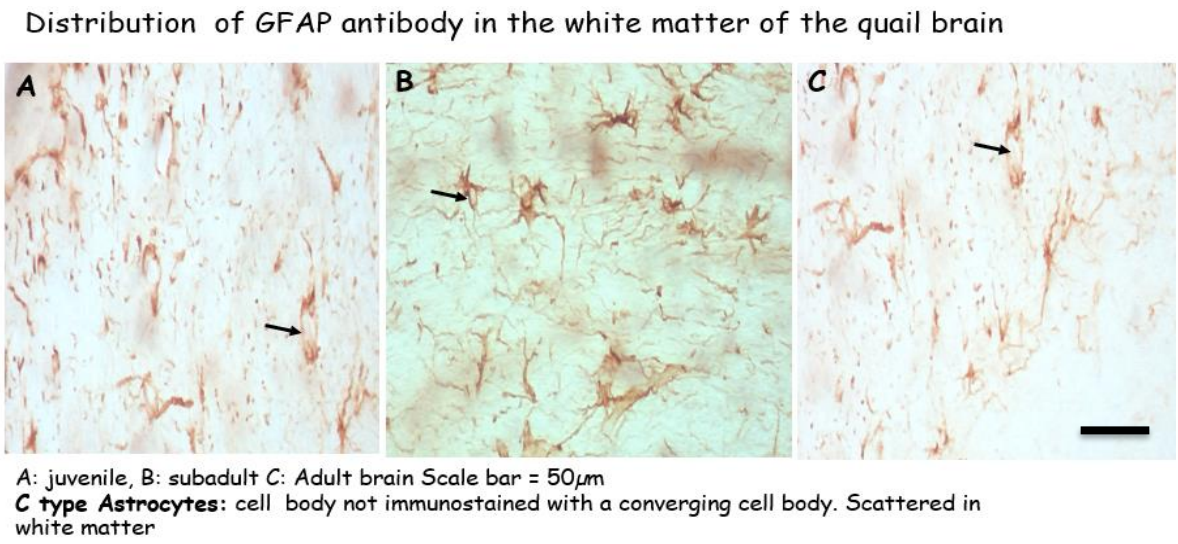


Figure 4.23: Photomicrograph of GFAP Type C immunopositive cells in the white mater, represented by the optic chiasma. Juvenile **(A)** and adult **(C)** brain both have moderate density of GFAP immunopositive cells which showed a typical characteristic of short processes, with somewhat empty soma. GFAP positive cells in the subadult exhibited the highest density. Scale bar: **C : 50µm**, applies to all

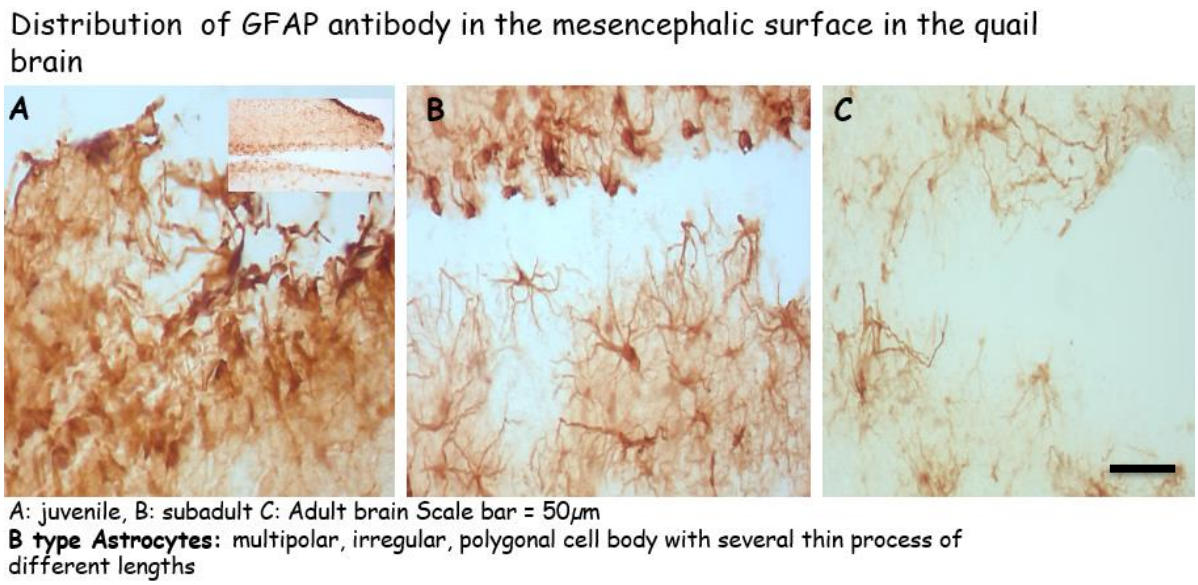


Figure 4.24: Photomicrograph of GFAP Type B immunopositive cells in the mesencephalic ventricle of the juvenile **(A)**, subadult **(B)** and adult **(C)** brain. They have darkly stained cell bodies and multipolar processes. GFAP positive cells in the juvenile and subadult showed the highest density while the adult brain has fewer moderate density. Scale bar : **C : 50µm**, applies to all

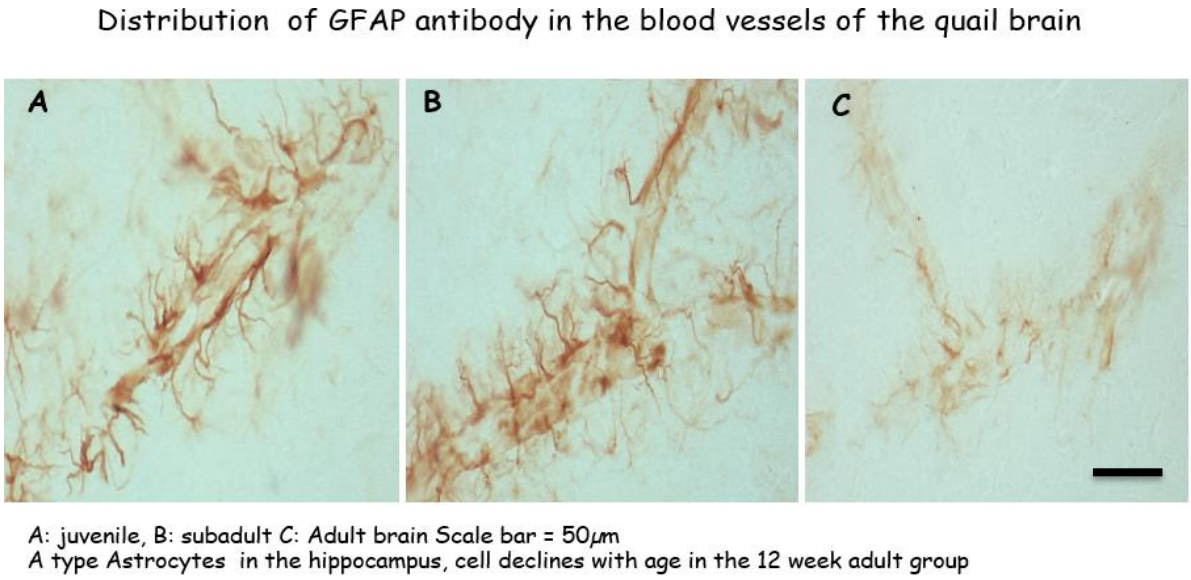


Figure 4.25: Photomicrograph of GFAP Type A immunopositive cells in the blood vessels of the juvenile **(A)**, subadult **(B)** and adult **(C)** brain. They are darkly stained body and processes. GFAP positive cells are absent in the core pallial regions but were positive for blood vessels. The adult blood vessels showed a very low density. Scale bar: **C : 50µm**, applies to all

4.6 Distribution and characterization of astrocytes identified by S100A1-ir cells in the quail brain.

4.6.1 Telencephalon

4.6.1.1 Pallium

S100A1-immunoreactive (S100A1-ir) cells were found to be absent in all the core pallial regions in the juvenile, subadult, and adult quail brain not restricted to the hyperpallium (H), mesopallium (M), nidopallium (N) and arcopallium (A) (Figure 4.26 A-C). However, high density of S100A1-ir cells were seen in the entopallium of the juvenile but decreased in the subadult and adult brain (Figure 4.26 A-F). In the juvenile brain, the L2 field demonstrated a moderate density and extended towards the lateral striatum but absent in the subadult and adult brain (Figure 4.26 A-C). S100A1-ir cells were identified in the hippocampus (HP), (APH) and piriform cortex (CP) (Figure 4.26 A-C). Across all experimental groups, the lateral ventricle of the ventricular zones was devoid of S100A1-ir elements, except at the medial border adjacent to the pallial surface (Figure 4.26 A-F).

4.6.1.2 Subpallium

The Subpallium exhibited a significant concentration of S100A1 immunoreactive cells (S100A1-ir) with the highest density observed in the globus pallidus (GP), predominantly associated with astrocytes and their extensive, interconnected processes with adjacent astrocytes (Figure 4.26 D-F). Astrocytes were more densely packed near the medial striatum (LSt), with both the cell body and nucleolus distinctly stained (Figure 4.26 A-C). A mix of astrocytes, glial elements and fibers was present. S100A1 immunoreactive cells astrocytes were more prevalent in juvenile and subadult brains

compared to adult brains which clearly showed a decline in density and processes in the adults (Figure 4.26 J-L).

A moderate density of S100A1-ir cells was found in the lateral striatum (LSt) and nucleus intrapeduncularis (INP), which were rich in glial elements in both juvenile and subadult quail. High density S100A1-ir cells and elements were scattered across the fasciculus prosencephali lateralis (FPL), tractus Quinto-frontalis (QF) (Figure 4.26 D-F) in all three experimental groups. Fiber tracts were more concentrated in juveniles and subadult (Figure 4.26 A-F) compared to the adult brains.

4.6.2 Diencephalon

In the epithalamus, the nucleus habenula has a moderate density of S100A1 immunoreactive cells and glia in the juvenile and subadult brain but low density in the adults (Figure 4.27 A-C). In the hypothalamus, the preoptic nucleus (POM), (PHN) and periventricular nucleus (PVN) are almost totally devoid of S100A1-ir cells (Figure 4.27 A-C) The median eminence (ME) and dorsal supraoptic decussation (DSD) showed a moderate density of glial element in the juvenile but remarkably low density in the subadult and totally devoid in the adult quail brain (Figure 4.27 A-C). In the thalamus, a population of S100A1-ir cells in the nucleus rotundus (RT) and (ICT) exhibited the highest density in the juvenile and subadult brain but decline in the adult (Figure 4.27 D-F). The cells within the nucleus rotundus were densely stained and only their cell body (Figure 4.27 G) there are moderately stained glia elements. The (ICT), unlike the (RT), is abundant in glial elements moderately stained (Figure 4.27 H), mostly an oligodendrocyte located adjacent to the (FPL) (Figure 4.27 I), and decline with age in the adult brain.

4.6.3 Mesencephalon

In the juvenile and subadult core mesencephalic area, the 3rd nerve nuclei, nVM SpM, SpL, nBor and OMN exhibited a high density of S100A1 immunoreactive cells (Figure 4.28 A-C) with moderate density in EW (Figure 4.28 D-F). These areas have a mixture of glia elements and neurons . A high density of positive astrocytes and oligodendrocytes were detected in some myelinated fiber tracts such as the posterior commissure, the ansa lenticularis, and the third nerve root both in panoramic and high magnification (Figure 4.28 A-C, I, K). The morphology of the cells seen in OMN showed astrocytes, oligodendrocytes and neurons with long processes (Figure 4.29 G-J). nVM are lined by another class of glia called tanycytes lined perpendicular to the nerve nuclei (Figure 4.29 D, 4.30 E), S100A1 immunoreactive cells in Nbor (Figure 4.29 F, 4.30 F) are neurons. In astrocytes, neurons, glia elements and fibers are clearly seen, well stained soma and nucleolus (Figure 4.29 G, 4.30 F-1), kissing astrocytes were found the juvenile mesencephalon (Figure 4.29 1) and (Figure 4.29 J) showed astrocytes with long processes and communicate with blood vessel. Ipc, Imc (Figure 4.29 A, M, 4.30 L) and SpM, SpL (Figure 4.29 A, K-L) which showed a moderate density of S100A1 glia immunoreactive cells. Other areas include nIII, EW, Mlad and FML (Figure 4.28, 29 30 A-F).

4.6.4 Cerebellum

In the juvenile cerebellum, the cerebellar layers showed a low density of S100A1 immunoreactive cells in the molecular cell layer (MCL) and Purkinje cell layer (PCL), while the granule cell layer (GCL), moderate density in subadult and adult brain (Figure 4.31 A-C). However, the subadult cerebellar layers exhibit a high density S100A1 immunoreactive cells in the PCL and GCL while the MCL is of moderate density. Similarly, the adult GCL showed high density but the PCL and MCL are of

moderate density S100A1 immunoreactive cells (Figure 4.31 A-C, H-1). Several medium-sized positive neurons were intensely stained in the nucleus cerebellaris internus (CBI) in both juvenile and subadult brain with a moderate density in the cerebellaris lateralis (CBI) (Figure 4.31 A-C, H-1). On the contrary, Low density S100A1 immunoreactive cells were localized in the adult brain. Mainly neurons and glia fibers were observed within the cerebellar cortex (Figure 4.31 A-1).

4.6.5 Pons and medulla

In general, the pons and medulla S100A1 immunoreactive cells were diffused within all the nuclei. However, there were some structures that are almost devoid of immunoreactive glial cells like (the locus coeruleus and the subcoerulean nuclei). High density of S100A1-ir cells glia cells was observed in some cranial nerve nuclei such as the nuclei of the VI and VII, the nucleus magnocellularis cochlearis, and the nucleus vestibularis dorsalis, and nucleus pontis lateralis and medialis (PL and PM), nucleus tractus descendens trigemini (TTD), nucleus reticularis pontis caudalis, pars gigantocellularis (RPgc), nucleus reticularis pontis caudalis (RP),in the superficial layers of the medulla (Figure 4.32 A-G). All the structures and nuclei mentioned are of high density of S100A1-ir cells both in glia and neuron in the juvenile quail (Figure 4.33 A1-6, B1-7). The subadult brains were of moderate density (Figure 4.34 A-L) while the adult brain declines and to some extent almost devoid (Figure 4.35 A-N). These neurons of the pontine and medullar regions were the biggest S100A1-ir cells observed along the whole quail brain. Immunopositive neurons are mainly located at the level of the nucleus motoris nervus trigeminus (MnV), nucleus reticularis (RP) and nucleus raphe (R), where they show small to big polygonal cell bodies bearing short processes. (FLM) fasciculus longitudinalis medialis; NV, nervus trigeminus; SCv,

nucleus subcoeruleus ventralis. S100A1-ir cells neurons within the nucleus motoris nervus trigeminus (MnV). nPrV, nucleus sensorius principalis nervi trigemini (Figure 4.32 A-G)**.** S100A1 Immunostained structures in the juvenile brain stem mostly stained cranial nerve nuclei and medulla (Figure 4.33 A1–A6, B1–B7), subadult (Figure 4.34 A-F) and adult (Figure 4.35 A, D-1) . Higher magnification showed glia element in Vem intertwine with fibers (Figure 4.33 A1–A2), astrocytes and neurons (Figure 4.33 A3–A6). The medulla nuclei showed neurons with their processes, astrocytes and glia elements in Rpc, Rpgc and VeL in the juvenile (Figure 4.33 B1–B9), moderate density in the subadult (Figure 4.34 G-L) and a low to devoid s100A1 immunoreactive cells in the adult brain (Figure 4.35 J-N),

Table 4.6 Summary of qualitative distribution and density of S100A1 immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of S100A1 immunoreactive structures

Brain Region			S100A1-ir cells		
			Juvenile	**Subadult**	**Adult**
Olfactory bulb	OBV				
Telencephalon	LV		++	++	-
	Pallium	HP	++	+	-
		APH	-	+	-
		CDL	-	+	-
		CPI	-	+	-
		HI	+	+	-
		HD	+	+	-
		M	-	+	-
		N	+	+	-
		NC	-	-	-
		E	-	+	-
	Subpallium	GP	+	+	+
		AC	++	+	-
		FPL	++	+	+
		SL	+++	++	+
		SM	+++	++	+
		QF	+	+	-
Diencephalon	Hypothalamus	POM	+++	++	+
	Epithalamus	Ha	+++	+++	+
		PVN	+++	++	+
		VMN	+	++	+
		PVN	+++	++	+
	Thalamus	Rt	+	-	++
		OV	+	-	-
		DLA	++	++	+
		DMA	++	++	+
		Glv	+++	++	+
Mesencephalon		OMN	++	++	+
		NBor	++	++	+
		DNIV	+++	++	+
		IPc	+	+	-
		IMc	+	+	-
		ME	++	+	+
Optic tectum		SO	++	+	+
		SGFS	++	+	+
		SGF	-	+	-
		SAC	+	-	+

	Optic tract	OT	++	+	+
	Optic chiasma	OC	+++	+++	+
	SLu	SLU	+++	+++	+
	Tectal ventricle	TV	-	+++	-
Rhombencephalon	Cerebellum	Cbi	-	-	-
		Ml	+++	-	-
		Pcl	+	-	-
		Gcl	-	-	-
	Pons	NVIIIc	+++	+	+
	Medulla		+	+	-
Other nuclei and		CA	++	+	-
Fibres		CT	++	+	-
		Ico	++	+	-
		FLM	+	++	-
		VeM	+	+	-
		VeL	+	+	-
		LoC	++	+++	+
		Mcc	-	+	-
		NVIII	+++	++	+

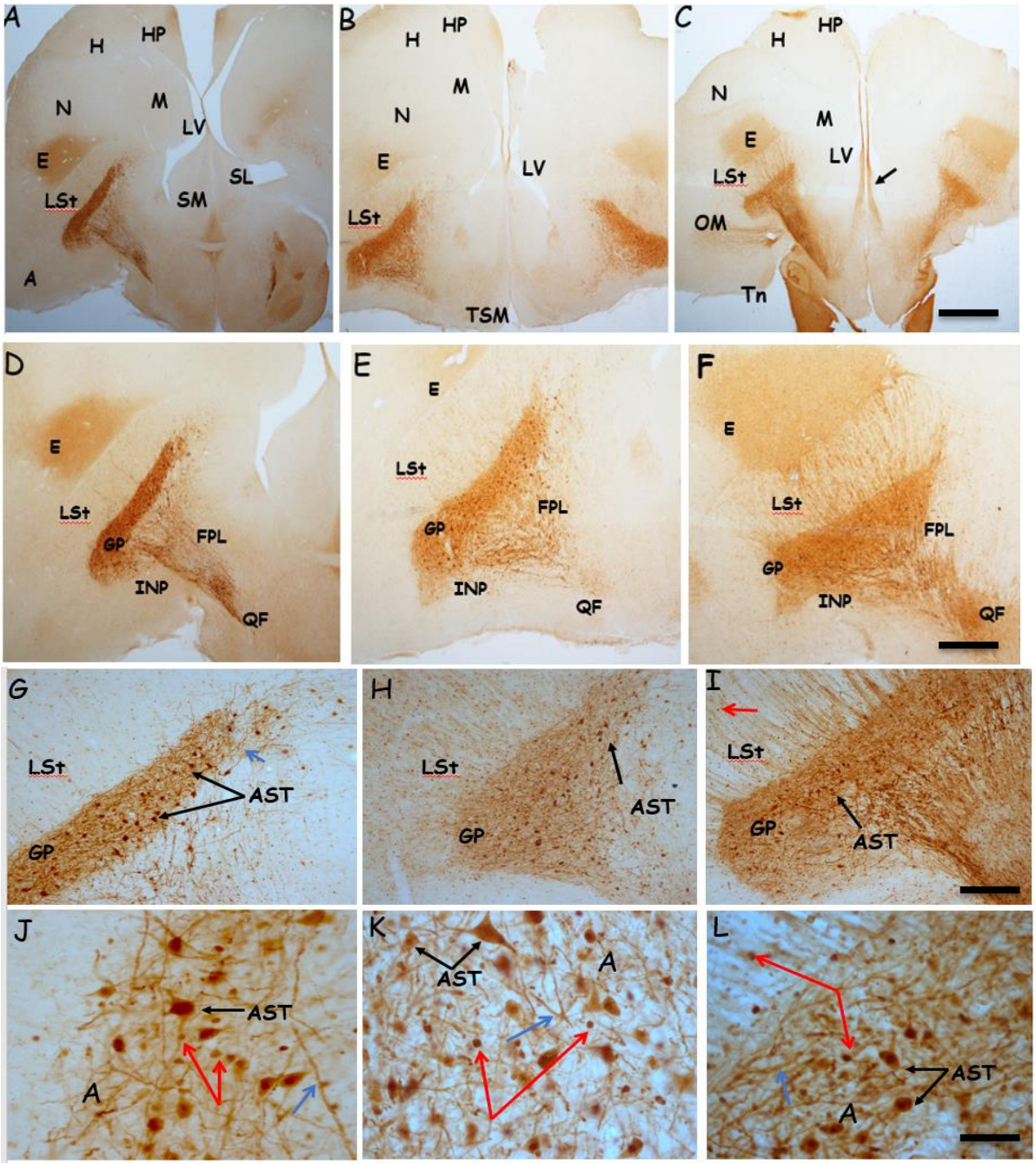


Figure 4.26: Photomicrographs of S100A1 Immunostained sections through the telencephalon. Panoramic views showed juvenile, subadult, and adult brain **(A–F)** showed glia elements in entopallium, hippocampus but absent in the core pallial area. Higher magnification **(G–L)** reveals a high density of S100A1 immunoreactive cells in the subpallium globus pallidus (GP) having all cell types**.** Red arrow: glial elements and astrocytes, blue arrow: astrocytic processes and fibers while black arrows indicate highly dense neuronal elements. The INP, QF and FPL typically show glial cell elements. Scale bars: **A–C** = 1 mm, **D-F** = 200µm, **G- I** = 500µm **J- L** = 50µm

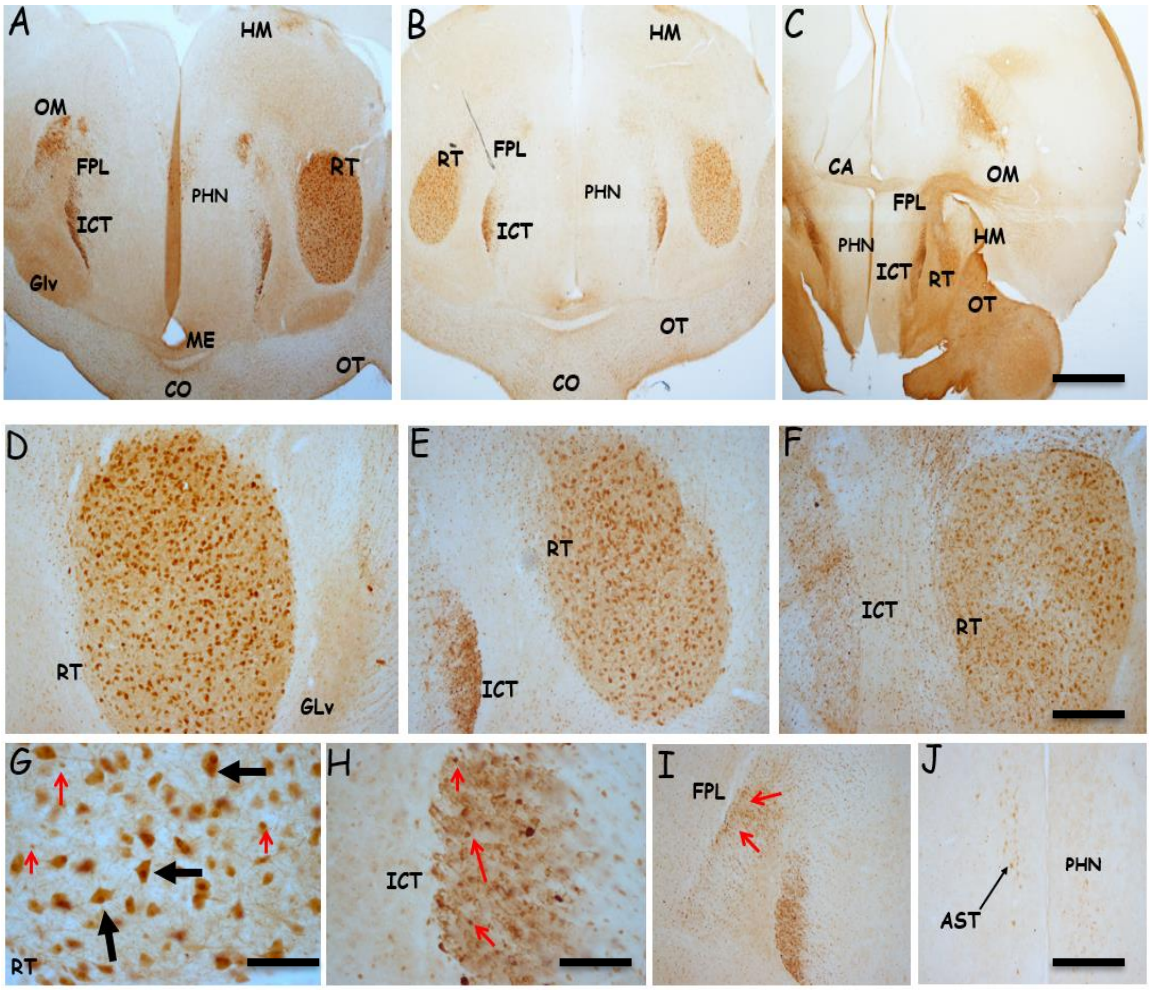


Figure 4.27: Photomicrographs of S100A1 Immunostained sections through the diencephalon. Panoramic views showed juvenile, subadult, and adult brain **(A–C)**. Higher magnification reveals a high density of S100A1 immunoreactive cells in the nucleus rotundus (Rt) **(D–F).** The cells are of high density in the juvenile **(D)** and decline as age progresses in the subadult **(E)** and adult **(F)** brain. Red arrows indicate glial elements, while black arrows indicate highly dense neuronal elements. The Rt and FPL typically show glial cell elements. In POM **(J)** there is low density of glia elements in the juvenile but almost devoid in the subadult and adult brain. Scale bars: **A–C** =1mm, **D, E,F, H, I J** = 500µm **G** = 50µm.

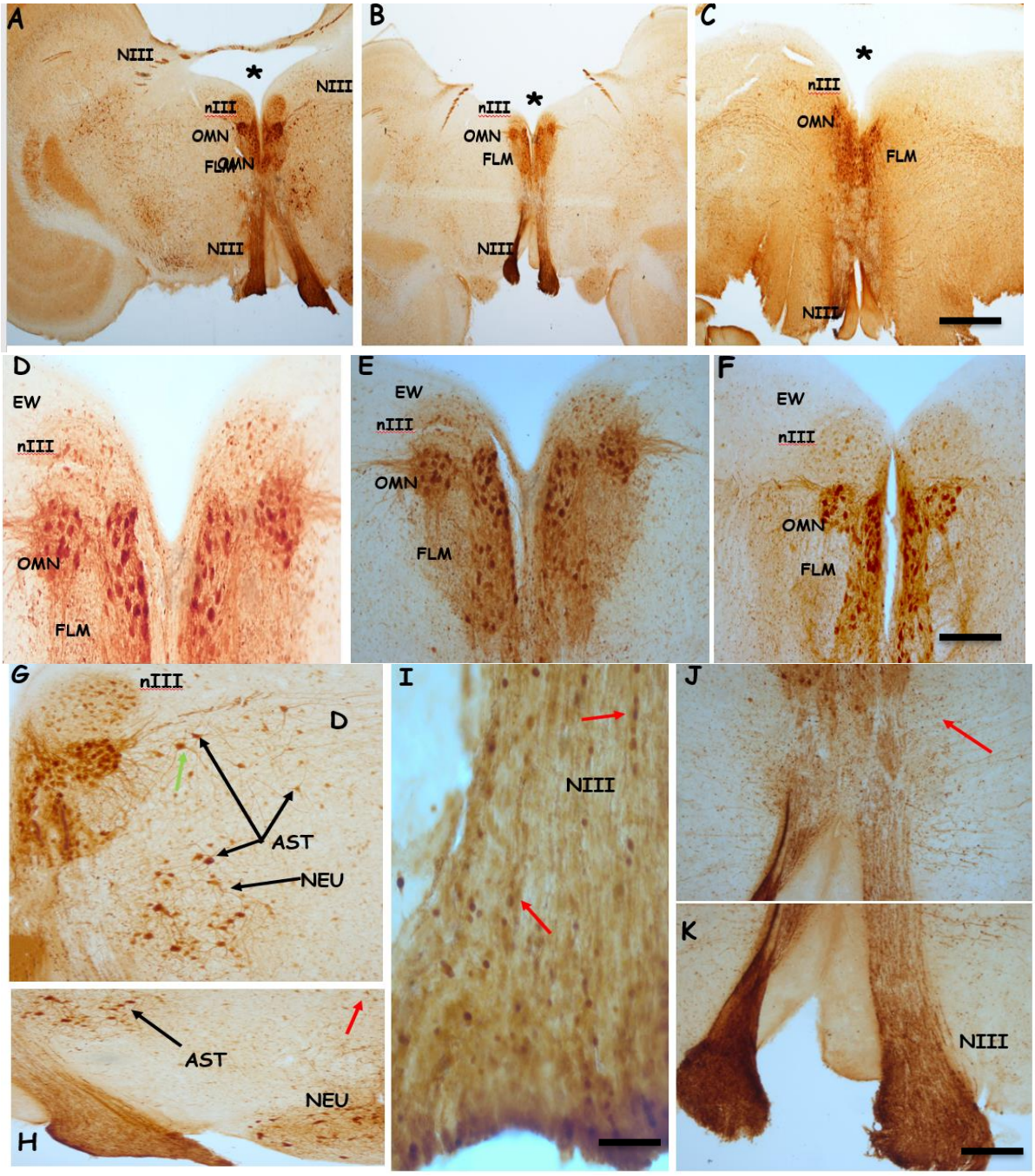

A
NIII
*
NIII
nIII
OMN
FLM
OMN
NIII
B
nIII
*
OMN
FLM
NIII
C
*
nIII
OMN
FLM
NIII
D
EW
nIII
OMN
FLM
E
EW
nIII
OMN
FLM
F
EW
nIII
OMN
FLM
G
nIII
D
AST
NEU
H
AST
NEU
I
NIII
J
K
NIII

Figure 4.28: Photomicrographs of the rostral mesencephalon in juvenile, subadult, and adult brain. Panoramic views showed juvenile, subadult, and adult brain **(A–C)**. Higher magnification reveals a high density of S100A1 immunoreactive cells in the nuclei (OMN), **(FLM), (nIII)** and **(NIII)** have a high density with a moderate density in **(EW) (A–C).** Higher magnification in **(D–F)** reveals glia, mostly astrocytes with processes in the oculomotor (OMN) and root of the 3rd nerve nucleus (J–K). The green arrow showed the morphology of tanycytes (G). Scale bars: **A–C** = 1 mm, **D–F, K** = 500µm, **G–H, I, J** = 50µm

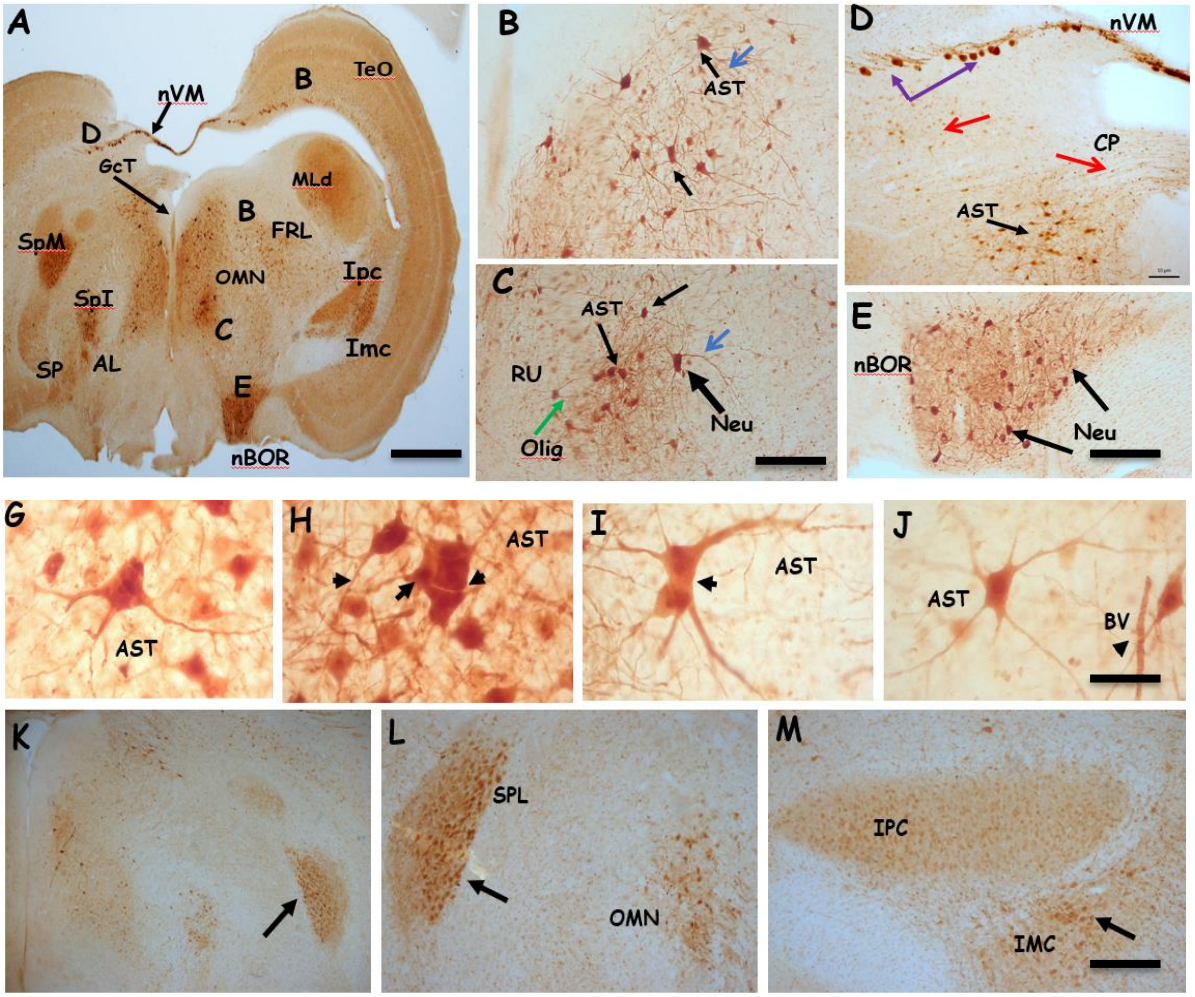


Figure 4.29: Photomicrographs of the middle mesencephalon exhibited the most intense and densely immunopositive S100A1 cells in the (SpM) and (SpL), (nBOR), (lpc), (lmc), (nVm) and Mlad in the juvenile brain but decline as age progresses in the panoramic view **(A).** Higher magnification in **(B–E)** showed: AST = Astrocytes (black and purple arrow) **(D)** = tanycytes (black arrow in B) Neurons. **(G–J):** Astrocytes, neurons, kissing astrocytes, blood vessels, and glial elements, red arrow in **(D)** is anterior commisure **(CA),** (lpc) and (lmc) showed both neuronal cell bodies and glia elements. Scale Bar: A = 1mm **B–E** = 500µm **G–J** = 50µm **K–M** = 200µm

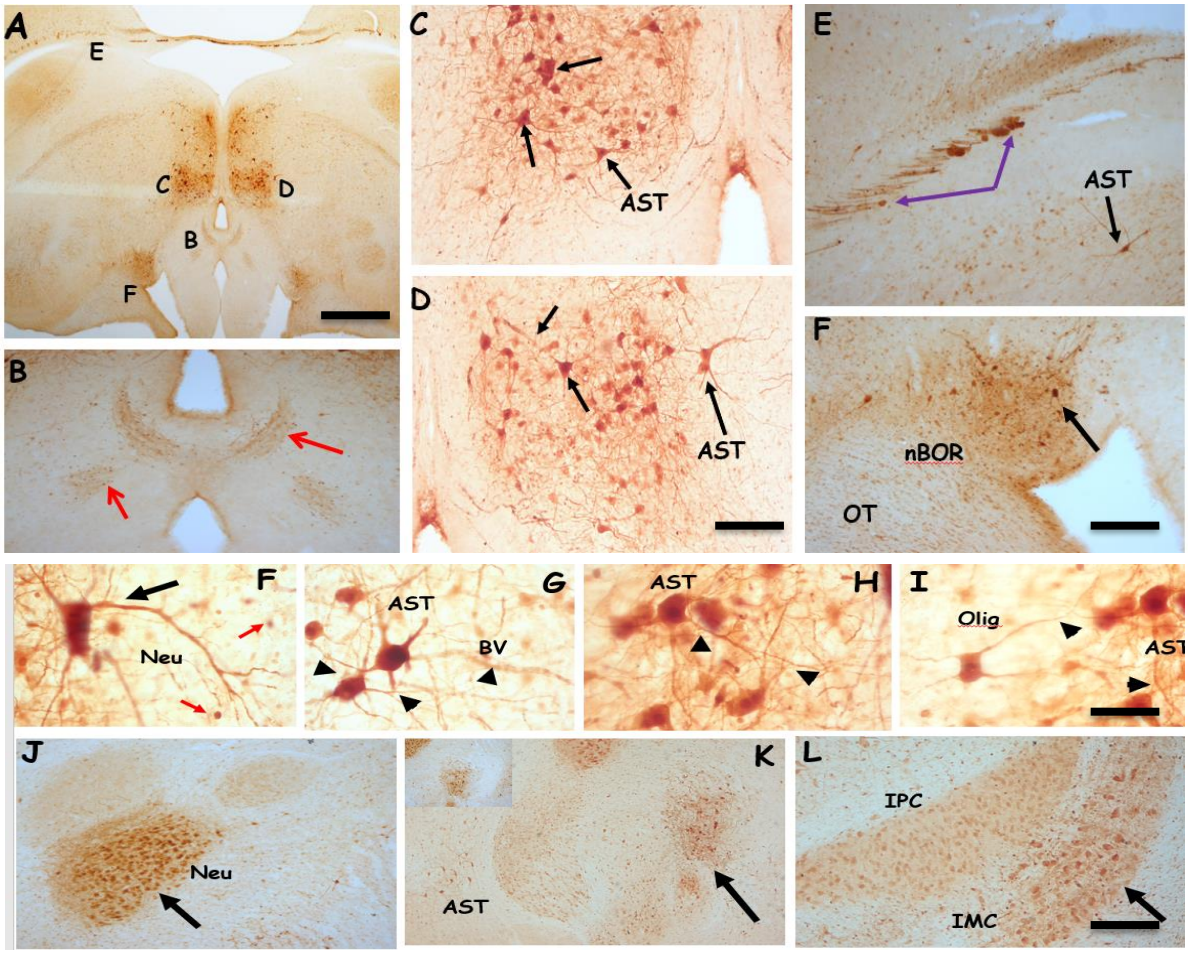


Figure 4.30: Photomicrographs of dorsal mesencephalon showed immunopositive S100A1 cells at the OMN, FLM which is intricate in both glia cells and neurons. Astrocytes oligodendrocyte and neurons **(F-I)** with all their mature morphological features in the juvenile brain. Higher magnification showed **(C)**: neurons and astrocytes, **(D):** kissing astrocytes and other astrocytes are seen, **(E):** tanycytes . Scale Bar: **A–B** = 1mm **C–F, L** = 500µm,

J, K=200µm, F-I =50µm

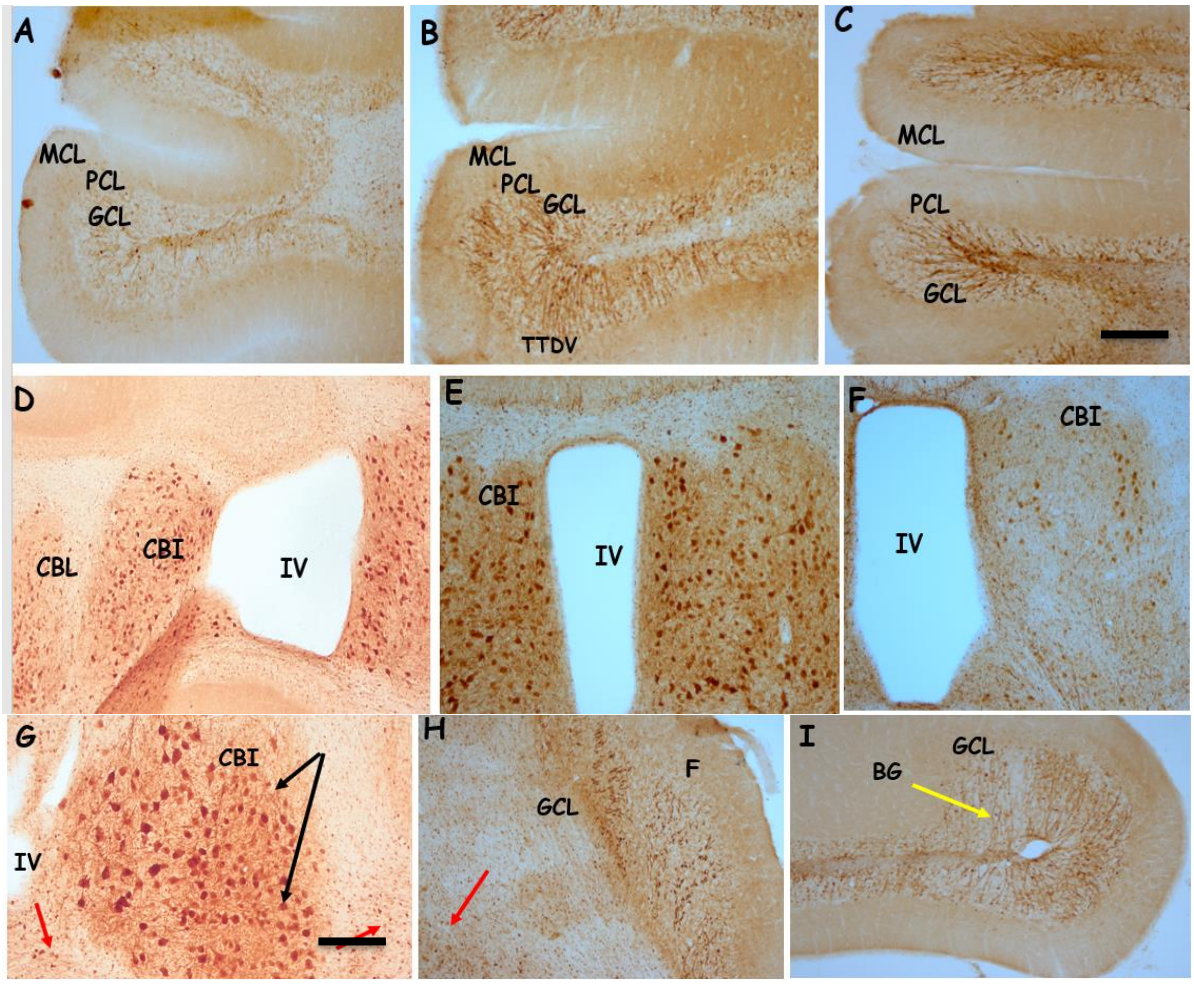


Figure 4.31: Photomicrograph through the cerebellum, panoramic view **(A–C)**. In the juvenile, subadult, and adult brain, S100A1-positive immunopositive cells were observed in the Purkinje, molecular, and granule cell layers **(D–F)**: High density neuronal immunopositive cells in CBI and CBL in juvenile and subadult but decline in adult. **(G)**: High magnification of CBI showed clusters of neuronal body (black arrow) while the layers are mostly of glia elements. **(H–I)**: Glial cells and fibers in the cerebellar layers. Scale Bar: **A–C** = 1mm **D–F, H–I** = 500µm, **G** = 50µm

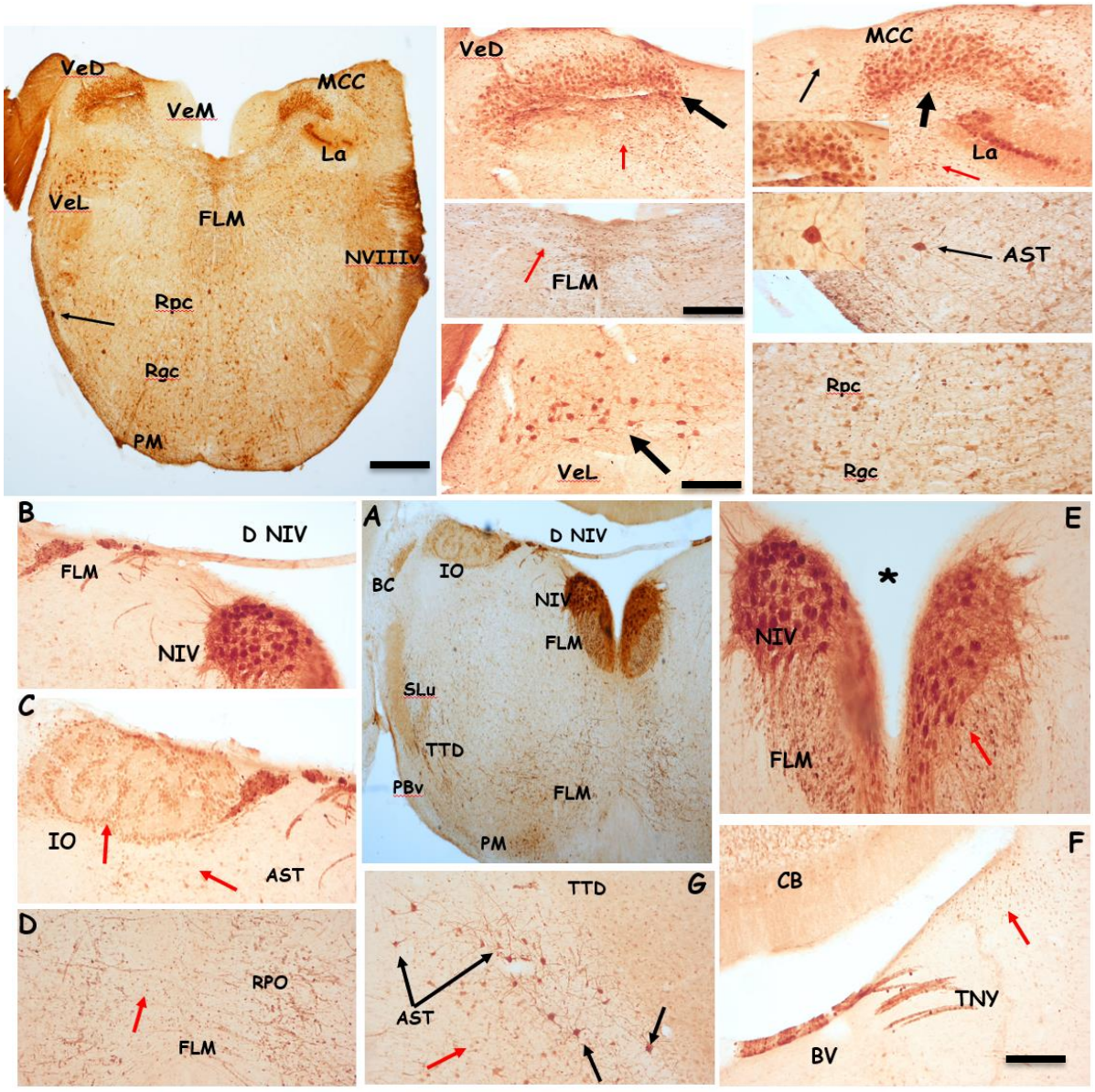
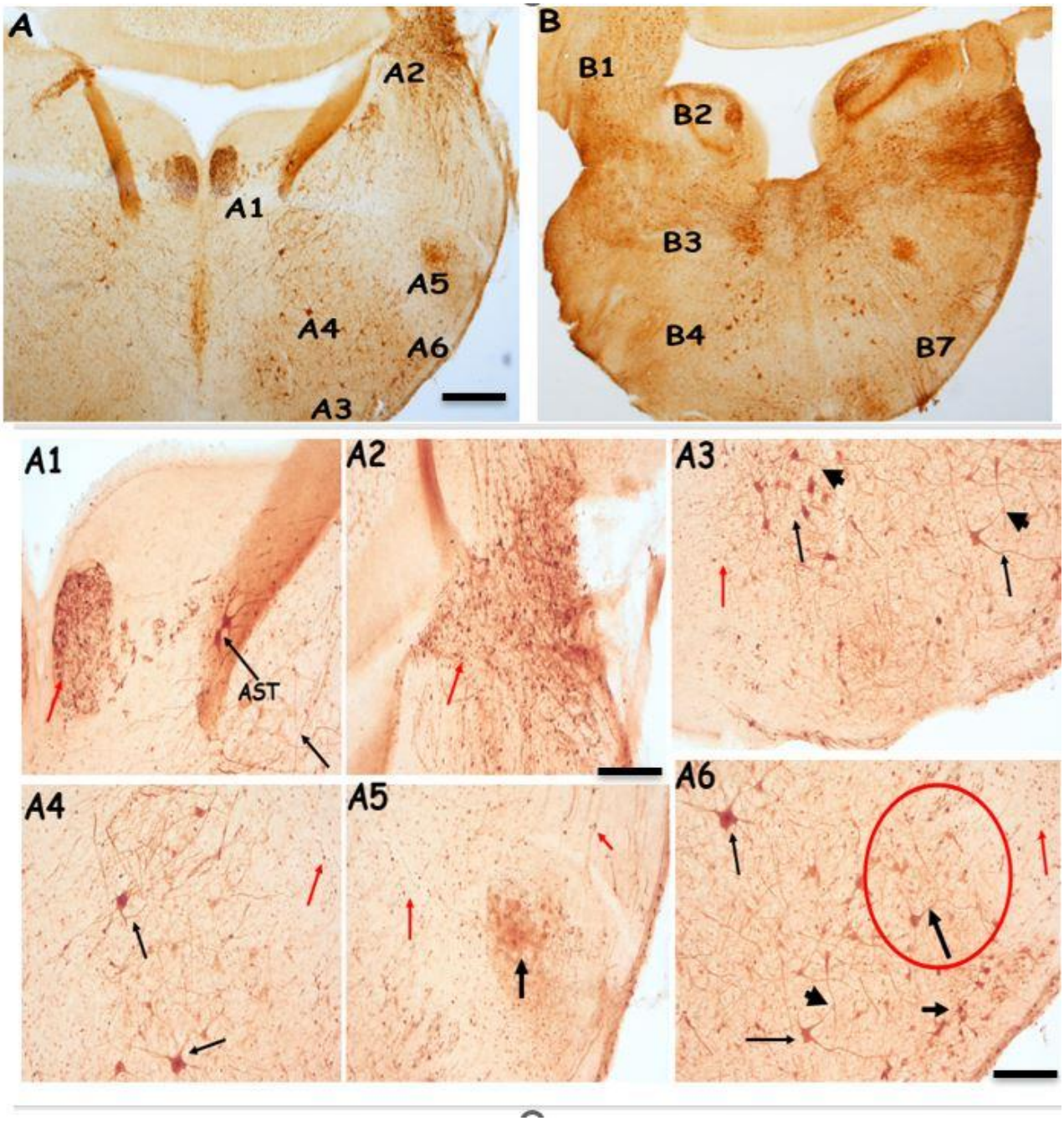


Figure 4:32. photomicrography of S100A1 protein Immunopositive structures in the pons and medulla; **A and B**: panoramic view of pons and medulla nuclei. Higher magnification showed: **D–F:** tanycytes, **D–G**: Black arrow: astrocytes, red arrow showed glia elements, nuclei nerve cells mostly neurons in Rpc, Rpgc and VeL. **Scalebar: A–B:**1mm, **C–F** = 500 µm


A
A1
A2
A3
A4
A5
A6
B
B1
B2
B3
B4
B7
A1
AST
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A5
A6

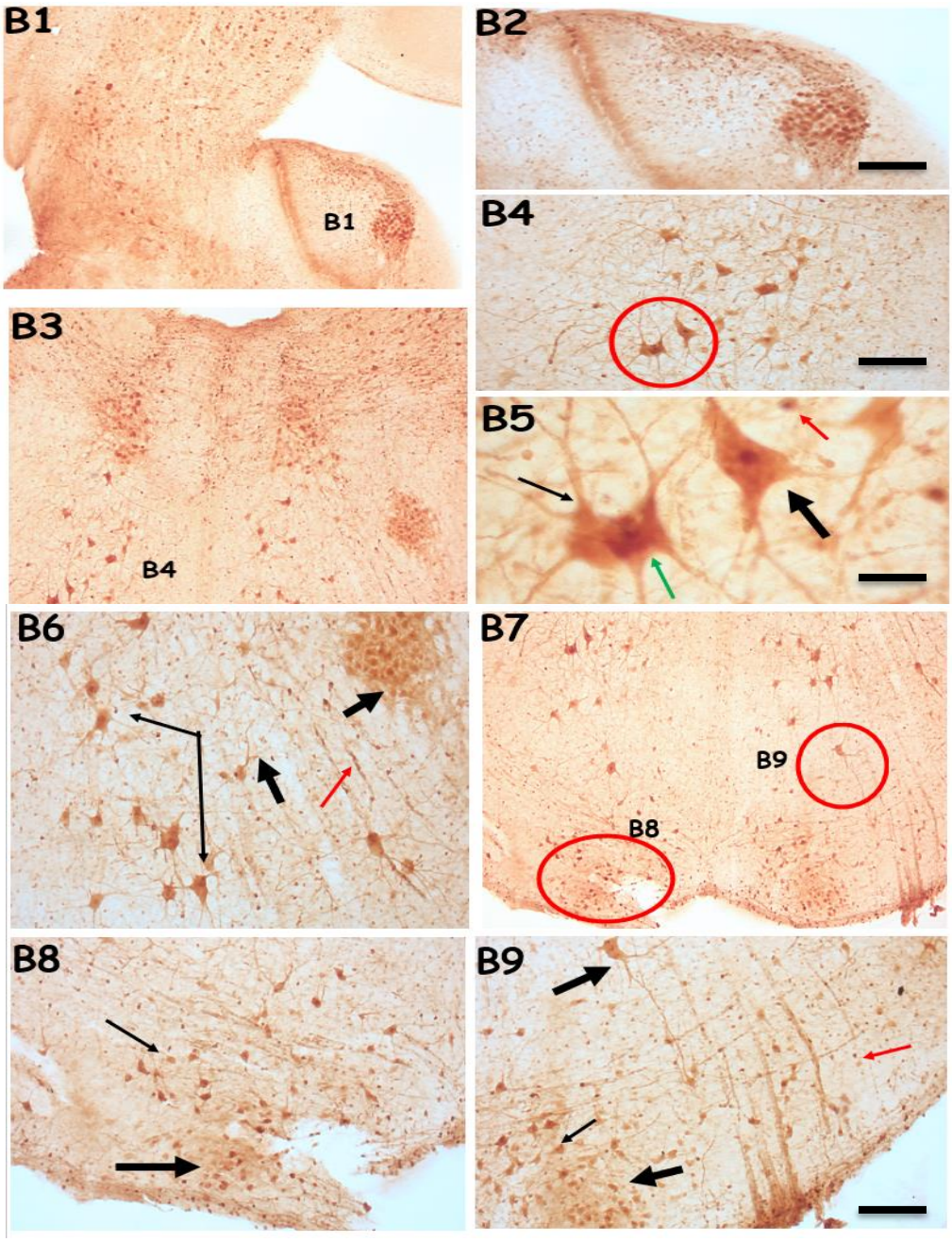
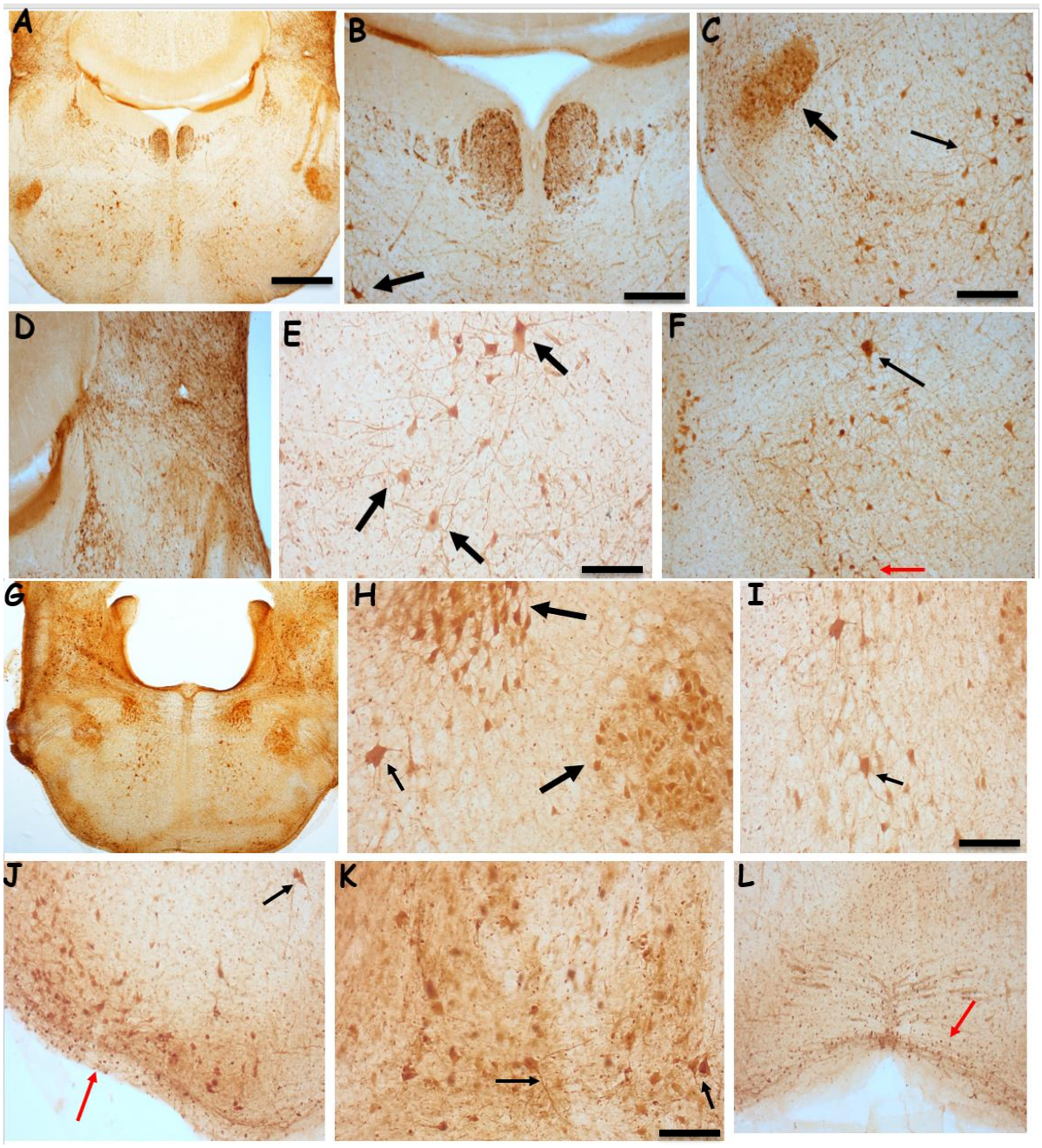
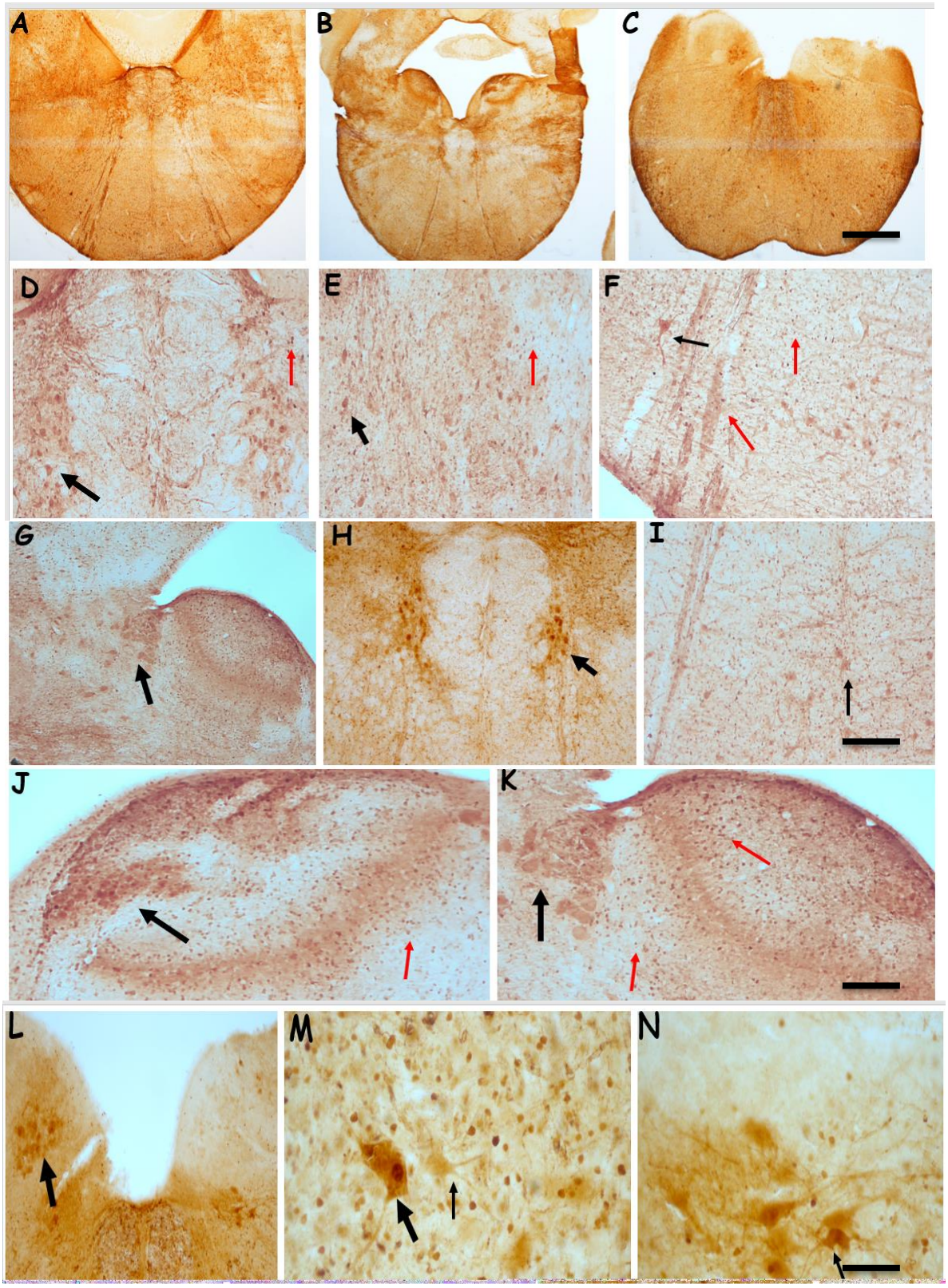


Figure 4:33. photomicrography of S100A1 Immunostained structures in the juvenile brain stem: mostly stained cranial nerve nuclei; panoramic view of pons (A) and medulla (B) showing all stained structures (**A1–A6) (B1–B7).** Higher magnification showed in **A1–A2:** glia element in **Vem** intertwine with fibers, **A3**: astrocytes, **A3**: astrocytes and neurons. **A4–A6** = higher magnification of **A1–A3.** For **B3, B7 & B8:** black arrows showed neurons with astrocytes and glia elements. B5: green arrow: oligodendrocytes, red arrow: glia element, fashion black arrow: astrocytes, blue black arrow: neuron with their processes. Rpc, Rpgc and VeL. **Scale Bar: A1–B1** =1mm, **A1–A6** = 500µm, **B3,4,7 & 8** = 500µm, **B5** = 50µm



4:34: photomicrograph of S100A1 Immunostained in the subadult brain stem; **(A–G):** panoramic view of pons (A) and medulla (G), there was reduced density, density is reduced compared to juvenile. Immunostained S100A1 positive cells are at **B.** OMN (glia elements) **(C),** mixed of glia elements (thick black arrow), and astrocytes indicated by the thin arrow; **(E–F & L)** showed a higher magnification showing the neurons and astrocytes (red arrow: astrocytes, black arrow: neurons) in in Rpc, Rpgc and VeL. **Scale Bar: A–G** = 1mm, **B, D, L, J**= 500µm, **C, E, F, H–I,K** =50µm


A
B
C
D
E
F
G
H
I
J
K
L
M
N

4:35: photomicrograph of S100A1 Immunostained in the adult brain stem; **(A–G):** panoramic view of pons (A) and medulla (G), there was reduced density, density is reduced compared to juvenile and subadult. The decline of immunopositive S100A1 positive cells are very pronounced, almost devoid at all pontine and medulla nuclei. **B.** OMN (glia elements) **(C),** mixed of glia elements (thick black arrow), and astrocytes indicated by the thin arrow; **(E–F & L)** showed a higher magnification showing the neurons and astrocytes (red arrow: astrocytes, black arrow: neurons). **Scale Bar: A–C**=1mm, **D**- L= 500µm, **M,N**=50µm

4.7 Distribution and characterization of oligodendrocytes identified by OLIG2-ir in the quail brain

Immunohistochemical analysis with the OLIG2-ir antibody revealed the distribution of oligodendrocytes across major brain divisions in juvenile, subadult, and adult male Japanese quails. Findings are presented by region, with developmental stages detailed within each.

4.7.1 Telencephalon

The telencephalon, encompassing the pallium and its subdivisions, exhibited widespread OLIG2-ir cell distribution with distinct patterns across developmental stages.

Pallium: In the experimental groups, OLIG2-ir cells were scattered throughout the pallium, primarily in gray matter, indicating early oligodendrocyte precursor cell (OPC) presence. In the juvenile and subadult groups, hyperpallium, mesopallium and entopallium exhibited a high density of OLIG2-ir cells. The nidopallium, nidopallium cortex and arcopallium showed a high to moderate densities of OLIG2-ir cells (Table 4.7). The cells are scattered within the pallium. The morphology is uniform in size and shape. However, the adult brain showed a decline in density in the pallium.

Subventricular Zone (SVZ): Juvenile SVZ contained sparse OLIG2-ir cells along the ventricular lining, consistent with a progenitor niche. Subadults exhibited a slight increase in OLIG2-ir cells, with some extending into adjacent pallial regions. In adults, OLIG2-ir cells were more prominent, indicating sustained oligodendrogenesis into maturity. In the juvenile quail brain, the ventricular zone exhibited a high density and concentration of OLIG2-ir. The ventral aspect of the lateral wall of the VZ exhibited a high density and concentration of OLIG2-ir, however the middle and dorsal aspect

of the lateral ventricular wall showed a moderate density of OLIG2-ir. The subadult brain has a moderate density while that of the adult brain showed a low density of OLIG2-ir cells.

Hippocampus (HP): OLIG2-ir cells were distributed across the hippocampal formation. In juvenile and subadult, the V area of the hippocampus has the highest density (Figure 4.36); dorsal area exhibited a moderate density. However, low densities of OLIG2-ir cells were observed in CDL, APH, and Cpi. The pattern of distribution is like those seen in juvenile and subadult, but there was a decline in the density of OLIG2-ir cells in the 12 weeks adult brain (Table 4.7).

Subpallium: OLIG2 immunoreactive cells OLIG2-ir were localized in the subpallial structures. In the juvenile brain, moderate densities of OLIG2-ir cells were exhibited in the lateral and medial striatum, medial and lateral septum. Highest density of OLIG2-ir cells were seen in the globus pallidus. The subadult and adult brain showed moderate and low densities in the above structures respectively.

Pial surface: the pial surfaces in the juvenile exhibit the most density OLIG2-ir cells than the subadult and adult brain. The entirety of the brain pial surface showed moderate and low densities in the subadult and adult brain.

White mater and fiber tracts: the white mater and fibers tracts in the telencephalon exhibit the highest density of OLIG2-ir cells which are not limited to the FPL, QF, TSM, CA (Figure 4.36) and CT in the juvenile but there is decline in both the subadult and adult groups.

4.7.2 Diencephalon

The diencephalon, including epithalamus, thalamic and hypothalamic regions, showed variable OLIG2-ir cell distribution. In the Juvenile and subadult quail, medial and lateral habenula showed a high density of OLIG2-ir cells while there was a decline in the adult brain. In the hypothalamus, areas such as POM, PVN, VMN and ME were observed to have a moderate density of OLIG2-ir cells in the juvenile but high density in the subadult as compared to the adult quail which exhibits low to absent in those areas (Figure 4.36).

The nucleus rotundus (RT), OV, DLA, DMA, and Glv showed a moderate density in the subadult but low density in the juvenile. However, the adult quail showed a decline in those areas

4.7.3 Mesencephalon and optic tectum

The mesencephalon, notably the optic tectum, exhibited consistent OLIG2-ir cells presence across stages.

Optic Tectum (TeO): layers of the optic tectum including SO and SGF were observed to have a high density in both Juvenile and subadult brain. The adult brain exhibits a high density of SO, but the SGF declines with age. However, SAC and SGFS were observed to decline in all the experimental groups. Areas such as Slu, IP, IM, OML and tectal ventricle exhibited a high density. Moderate density of OLIG2-ir cells were observed in OMN, MLD, IP, IM, and nBOR. White mater and fiber tracts including FML, optic chiasma, optic tract and its decussation also showed a high density in the juvenile. All structures decline as development continues in the subadult and adult quail brain.

4.7.4 Cerebellum

The rhombencephalon, including the cerebellum, displayed significant OLIG2-ir cell distribution, particularly in motor-related areas.

In juveniles, OLIG2-ir cells were abundant throughout the cerebellum, scattered or sparsely distributed in gray matter with moderate white matter presence near tracts. OLIG2-ir cells are also present in the Purkinje, molecular and granular cell layers. Subadults showed an increased OLIG2-ir cells, with aggregation along cerebellar white matter (WM), reflecting differentiating oligodendrocytes (Table 4.7). Adults exhibited dense OLIG2-ir clustering in white matter tracts (cerebellar peduncles), consistent with extensive myelination for motor coordination.

In the juvenile brain, MCC, VED, La, IO, LoC. OLIG2-ir were observed in most of the cranial nerve nuclei and not limited to N1X, MNX, NVI, NVIII, NIII with the highest density in the juvenile as compared to the subadult and adult brains (Table 4.5).

Table 4.7 Summary of qualitative distribution and density of OLIG2 immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of OLIG2 immunoreactive structures

Brain Region		OLIG2-ir cells			
			Juvenile	Subadult	Adult
Olfactory bulb	OBV	TSM	+++	+	__
Telencephalon	LV	SVZ	+++	++	+
	Pallium	HP	+++	++	+
		APH	++	++	+
		CDL	++	++	+
		CPI	++	++	+
		HA	+	_	_
		HI	+	_	_
		HD	+	_	_
		M	+	_	_
		N	+	_	_
		NC	+	_	_
		E			
		PS	+++	++	+
		A	++	__	__
	Subpallium	Mst	++	+	_
		Lst	++	+	_
		GP	+++	++	+
		SL	++	_	_
		TN			
		SM	++	_	_
		BSTM			
		BSTL			
Diencephalon	Hypothalamus	POM	++	++	+
		PVN	++	++	+
		VMN	++	++	+
		Tu			
	Thalamus	Rt	+	++	+
		OV	+	++	+
		DLA	+	++	+
		DMA		++	+
		Glv	++	++	+
Mesencephalon		Gct			
		OMN	++	+	
		RMC			
Optic tectum		SO		+++	++
		SGFS		+	
		SGF		+++	++
		SAC		+	_
Rhombencephalon	cerebellum	Cbi	+++	__	__
		Ml			
		Pcl			
		Gcl			
	Pons	NVIIIc			

	Medulla oblongata				
		CA	+++	+++	++
		QF	+++	+++	++
		OPHC T	+++	+++	++
		OPCH C	+++	+++	++
		GSD	+++	+++	++
		RT	+++	+++	++
		TPL	+++	+++	++
		CP	+++	+++	++
		LH	+++	+++	++
		MH	+++	+++	++
		VZ	+++	+++	++
		FML	+++	+++	++
		CT	++	++	+
		HPF	++	++	+
		NCL	++	++	+
		POA	++	++	+
		MLD	+	+	
		IO	++	++	+
		SLU	+++	+++	++
		IP	+++	+++	++
		IM	+++	+++	++
		VTILING	+++	+++	++
		TECTAFR	+++	+++	++
		OML	+++	+++	++
		NBUR	++	++	+
		NILL	++	++	+
		CT	++	++	+
		SGF			
		DTN	+	+	
		SFG	+	+	
		LV	+	+	
		VED	++	++	+
		MEC	++	++	++
		ME	+	+	
		OT			
		OC			
		TEO			
		TRO			
		SPL	+	+	
		SPM	+	+	
		OPCH TEC	+++	+++	++
		DCUSS	+++	+++	++

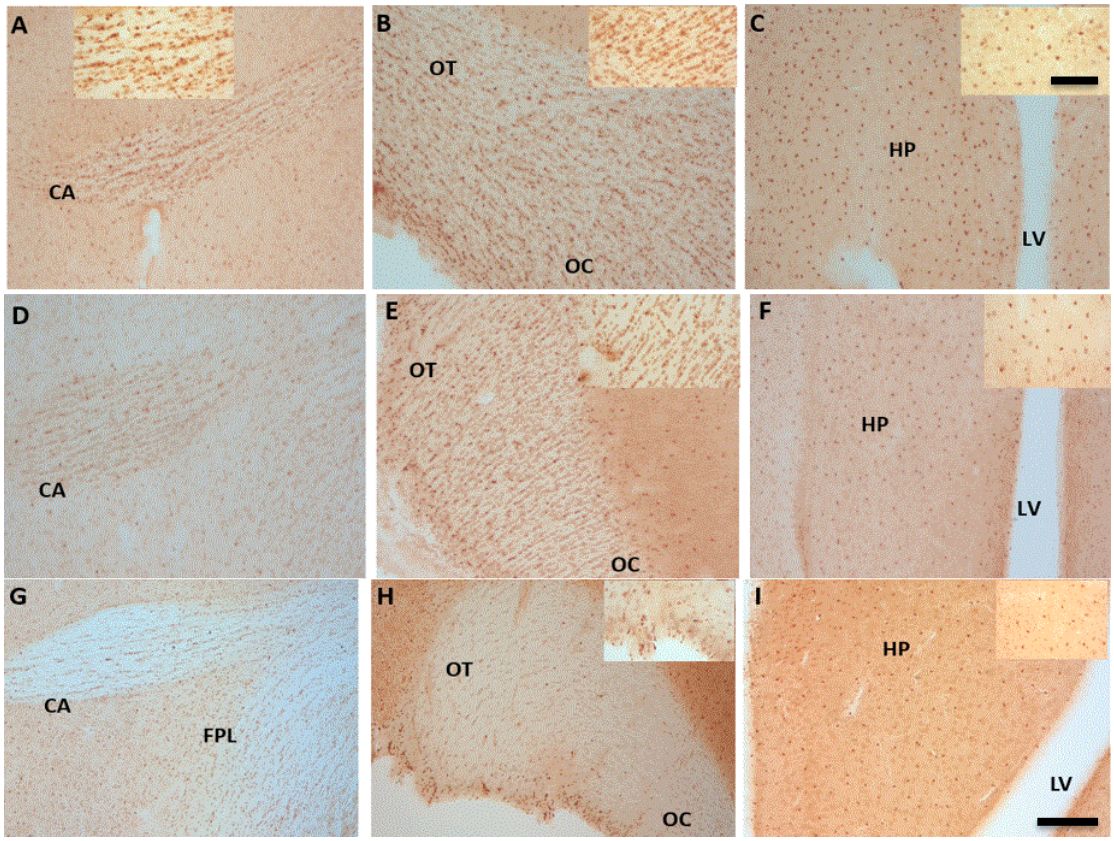


Figure 4.36: photomicrograph of Olig2 immunoreactive cells in the white matter of (C) Fraternal (CA) commissure in the juvenile, subadult and adult quail brain (CA, G) (BE, H) optic tectum (OT) and (C, F, I) the hippocampus (AD) show the classical oligodendrocytes, arranged in rows - multiple OLIG2 immunopositive cells are of high density in the juvenile brain and subadult brain but decline in the adult brain. The inserted photomicrographs are of higher magnification. In the CA and OT, they are congested and aggregated while in the hippocampus they are scattered. Scale bar: A–I = 500µm (for inserted photo 50µm)

4.8 Distribution of IBA-1 immunoreactive cells in the quail brain

4.8.1 Olfactory bulb

IBA-1 immunoreactive cells (IBA-1-ir) were absent in the olfactory bulb of Japanese quail across all age groups.

4.8.2 Telencephalon (Pallium, and Sub pallium)

The lateral ventricle (LV) region demonstrated a high density of IBA-1-ir cells in juveniles, moderate in subadults, and absent in adult Japanese quails. The HP region revealed a moderate density in juvenile quails, low density in subadults, and absence in adult quails (Figure 4.37 D-1). The APH, CDL, CPI, and M regions were present in small quantities in the subadults but were absent in both juvenile and adult quails (Figure 4.8) . In the HA, HI, HD, N, and E regions, the density was observed to be small in density in the juvenile and the subadult quails but were observed to be absent in the adult quails. In the E region, IBA-1-ir cells were moderate in juvenile and subadult brains but absent in the adult brain (Table 4.8). The anterior commisure has a high density of IBA-1-ir cells in subadults mostly transitional microglia (Figure 4.38 B, E), juvenile has moderate density of IBA-1-ir cells mostly amoeboid with processes (Figure 4.38 A, D), adult which devoid of IBA-1-ir cells (Figure 4.38 C).

4.8.3 Diencephalon (thalamus and hypothalamus)

The thalamus regions (DMA, Glv and DLAI) both demonstrated a high density of IBA-1-ir cells in both juveniles and subadults (Table 4. 8), in these regions, the cells had an amoeboid-like appearance without ramified branching processes, which were present in low density in the adult quails; the hypothalamus regions of the

paraventricular nucleus and ventromedial nucleus (POM, PVN and VMN) all had moderate density of IBA-1-ir cells in the subadult group (Figure 4.39 B, E), low density in the juvenile (Figure 4.39 A, D) and very low to almost devoid in the adult quail (Figure 4.39 E).

4.8.4 Mesencephalon and optic tectum

The mesencephalic region of OMN had a moderate density of IBA-1-ir cells in both juveniles and subadults, but a low density was observed in adult quails (Table 4.8). The optic tectum regions SO and SGF had a high density of IBA-1-ir cells across all age groups. However, in the SAC region, there was little density of IBA-1-ir cells across all age groups of the Japanese quail (Table 4.8).

4.8.5 Rhombencephalon (cerebellum, pons, and medulla)

The nucleus semilunaris (SLu) has the highest density and aggregation of IBA-1 immunoreactive cells in the juvenile and subadult but devoid of processes (amoeboid microglia) but decline in the adults (Figure 4.39 A-C). The cerebellum molecule (Ml) had a high density of IBA-1-ir cells in the juvenile but was absent in both the subadult and adult groups, while the Purkinje cell layer (Pcl) and granular cell layer (Gcl) layer had a low density of IBA-1-ir cells in the juvenile but was absent in the subadult and adult groups (Table 4.8). However, only adult granular cell layer (Gcl) showed a mild distribution of IBA-1-ir cells. The cells exhibited a spindle-like structure with long branching processes, with each process connected to adjacent processes. IBA-1-ir cells were concentric across brain regions and traversed from one layer of the cerebellar

cortex to another (from the granular layer to the Purkinje cell layer). None of these cells were observed in the cerebellar white matter layer. In the pons, there was an observable high density of IBA-1-ir cells in the juveniles and a moderate density in the subadults but absent in the adult group (Table 4.8).

Table 4.8 Summary of qualitative distribution and density of IBA-1 immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of IBA-1 immunoreactive structures

Brain Region			IBA-1-ir cells		
			Juvenile	Subadult	Adult
Olfactory bulb	OBV				
Telencephalon	LV		++	++	-
	Pallium	HP	++	+	-
		APH	-	+	-
		CDL	-	+	-
		CPI	-	+	-
		HI	+	+	-
		HD	+	+	-
		M	-	+	-
		N	+	+	-
		NC	-	-	-
		E	-	+	-
	Subpallium	GP	+	+	+
		AC	++	+	-
		FPL	++	+	+
		SL	+++	++	+
		SM	+++	++	+
		QF	+	+	-
Diencephalon	Hypothalamus	POM	+++	++	+
	Epithalamus	Ha	+++	+++	+
		PVN	+++	++	+
		VMN	+	++	+
		PVN	+++	++	+
	Thalamus	Rt	+	-	++
		OV	+	-	-
		DLA	++	++	+
		DMA	++	++	+
		Glv	+++	++	+
Mesencephalon		OMN	++	++	+
		NBor	++	++	+
		DNIV	+++	++	+
		IPc	+	+	-
		IMc	+	+	-
		ME	++	+	+
Optic tectum		SO	++	+	+
		SGFS	++	+	+
		SGF	-	+	-

		SAC	+	-	+
	Optic tract	OT	++	+	+
	Optic chiasma	OC	+++	+++	+
	SLu	SLU	+++	+++	+
	tectal ventricle	Tec V	++	+++	-
Rhombencephalon	Cerebellum	Cbi	-	-	-
		Ml	+++	-	-
		Pcl	+	-	-
		Gcl	-	-	-
	Pons	NVIIIc	+++	+	+
	Medulla		+	+	-
Other nuclei and		CA	++	+	-
Fibres		CT	++	+	-
		Ico	++	+	-
		FLM	+	++	-
		VeM	+	+	-
		VeL	+	+	-
		LoC	++	+++	+
		Mcc	-	+	-
		NVIII	+++	++	+

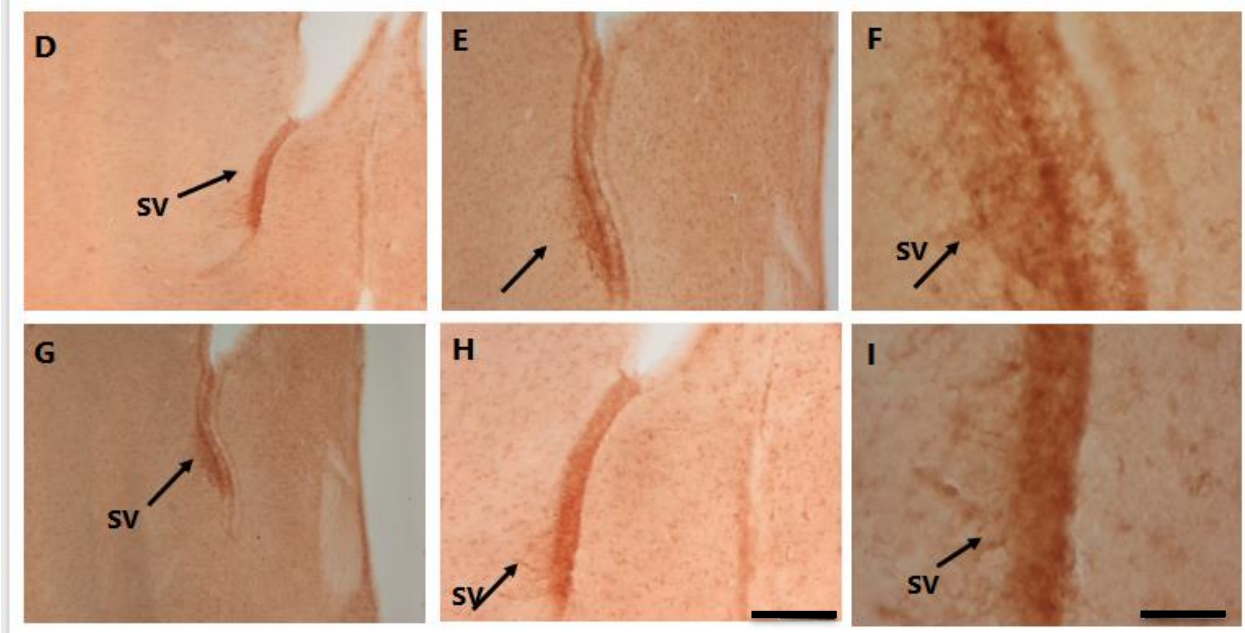


Figure 4.37: Photomicrograph of the distribution of IBA-1 immunopositive cells observed in the rostral ventricular part of the lateral ventricle in the juvenile **(D-G)**, subadult **(E-H)** and adult **(F-I)** quail brain. Arrows showed high density in all experimental groups. Higher magnification of IBA-1 immunoreactive cells **(F, I).** Scale bar = **D,E,G,H** 500µm. **F,** 50µm.

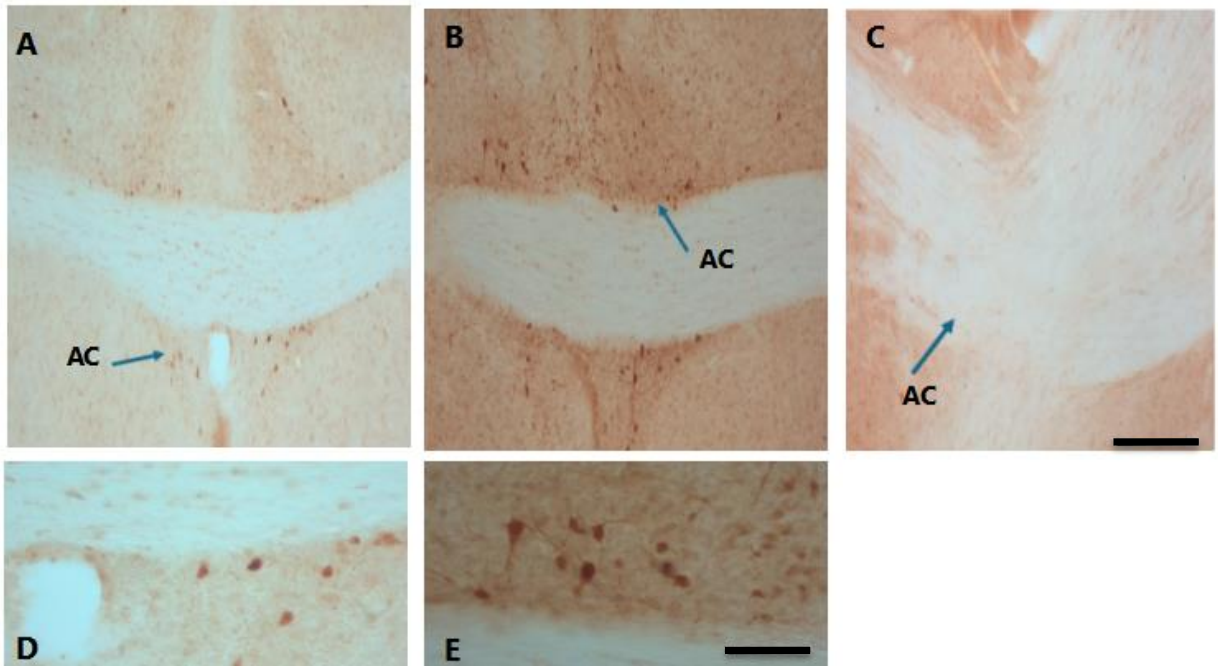


Figure 4.38 Photomicrograph of IBA-1 immunoreactive cells in the anterior commisure: juvenile **(A)** showed a low few positive cells, amoeboid type of microglia **(B)** subadult microglia mostly transitional microglia with short processes and high density in the soma. IBA-I is totally absent in the adult brain **(C)** higher magnification seen in (D-E) juvenile: Scale bar: **A-C** = 500µm, **D-E** = 50µm.

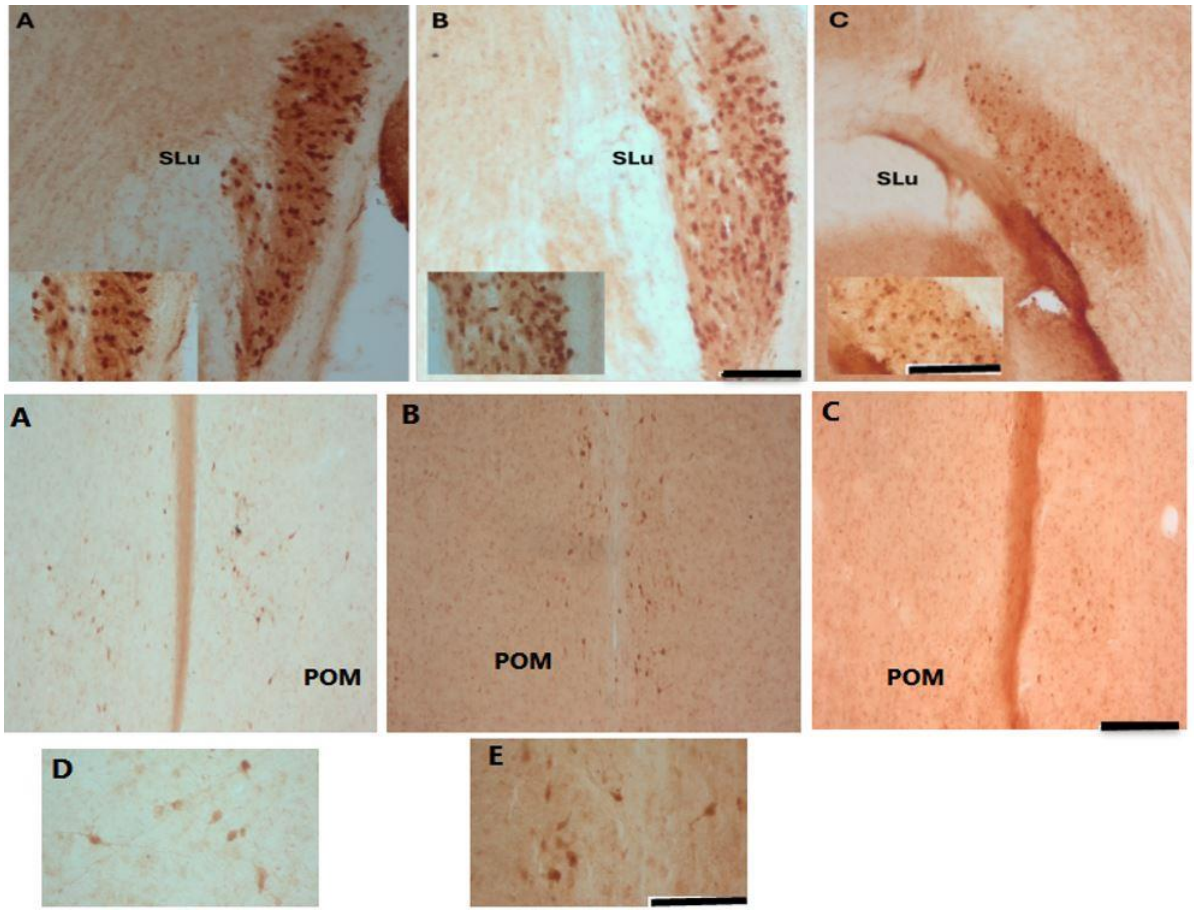


Figure 4.39: Photomicrograph of IBA-1 immunoreactive cells in the nucleus semilunaris **(SLu)** with a high density aggregated IBA-1 immunoreactive cells, devoid of processes (amoeboid microglia) in the juvenile **(A),** subadult **(B)** and adult **(C).** Higher magnification photomicrograph in subadult and adult were seen to decline with age. There was a low density of IBA-1 immunoreactive cells in the adult **(C)** preoptic nucleus **(POM)** with moderate density in subadult and low density in the juvenile brain **(A-D)** moderate density in subadult **(B-E)** and almost devoid in adults. Scale bar: **C** = 500µm, insert **A-C D, E** = 50µm.

4.9 Distribution of DCX immunoreactive cells in the quail brain

The telencephalon exhibited the highest concentration of DCX immunoreactive cells (DCX-ir) and fibers, with density differences observed across various pallium areas. In the brains of juvenile and subadult quail, the HA region displayed a notable presence of DCX-ir cells in both medial and lateral sections, while the core regions exhibited a lower density (Figure 4.41). This density diminished in a rostro-caudal direction towards the anterior commissure. The HD and HI regions showed a high density of DCX-ir cells, receiving neuroblasts from the dorsal lateral ventricle. A significant density of DCX-ir fibers was observed in pallial regions adjacent to the lateral ventricles, with the highest concentration in M and Mst, which decreased rostro-caudally towards CA. The density of DCX-ir cells in nidopallium N varied along the rostro-caudal axis. The nidopallium frontale exhibited a moderate density at rostral levels, with higher concentrations in medial and lateral regions but sparse in core areas. Neuroblasts extended from the lateral ventricle VZ into the parenchyma (Figure 4.40). The nidopallium showed a low density of DCX-ir cells, with none present in the core region.

The NC had the highest density of DCX-ir cells near the lateral ventricle VZ. DCX-ir elements were absent in the entopallium and arcopallium across all experimental groups. The hippocampus (HP) and the anterior parahippocampal area (APH) exhibited the lowest density of doublecortin-immunoreactive (DCX-ir) cells within the pallium of both juvenile and subadult brains (Table 4.9). In contrast, a moderate density of DCX-ir cells, lacking processes, was observed in the adult brain. The central dorsal lateral (CDL) and central posterior intermediate (CPi) regions demonstrated a high density of DCX-ir cells across juvenile, subadult, and adult brains. In the juvenile brain, DCX immunoreactive cells were found in low numbers in the medial hypothalamus near the SVZ of the third ventricle.

The dorsolateral thalamic nuclei, which include DMA, DLL, and DLM, were present only in juvenile brains and not in subadult or adult brains. No DCX immunoreactive cells were detected in the ventricular or parenchymal regions of the mesencephalon and optic tectum at any age (Table 4.9). In the cerebellar cortex of juvenile and subadult quail, DCX-ir fibers were densely packed in the Purkinje layers, but this specific staining was absent in adult brains. Although the pattern of DCX-ir cells in the cerebellar cortex of juvenile and subadult quail was similar, their density decreased with age. At no age were DCX-ir cells observed in the Cbi (Figure 4.42).

Table 4.9 Summary of qualitative distribution and density of DCX immunoreactive structures in the juvenile, subadult and adult Japanese quail brains

- absent + low ++ moderate +++ high density of DCX immunoreactive structures

Brain Region			DCX-ir cells		
			Juvenile	Subadult	Adult
Telencephalon	LV		+++	+++	++
	Pallium	HP	++	++	+
		HD			
		E			
		N	++	++	++
		M	++	++	++
		VZ	+++	+++	++
		SVZ	+++	+++	++
	Subpallium	Mst	+++	+++	++
		Lst	+++	+++	++
		GP	+++	+++	++
		SL	+++	+++	++
		SM	+++	+++	++
		BSTM	+++	+++	++
		BSTL	+++	+++	++
Diencephalon	Hypothalamus	POM	+	+++	+
		PVN	+	+++	+
		VMN	+	+++	+
		Tu	-	-	-
	Thalamus	Rt	+++	++	+
		OV	+++	++	+
		DLA	+++	++	+
		DMA	+++	++	+
		Glv	+++	++	+
Mesencephalon		Gct	++	+	
		OMN	+	+	
Optic tectum		SO	+++	++	+
		SGFS	++	++	+
		SGF			
		SAC	+++	++	+
Rhombencephalon	cerebellum	Cbi			
		Ml	-	-	-
		Pcl	+++	++	+
		Gcl	-	-	-
	Pons	NVIIIc	+++	+++	+
	Medulla				

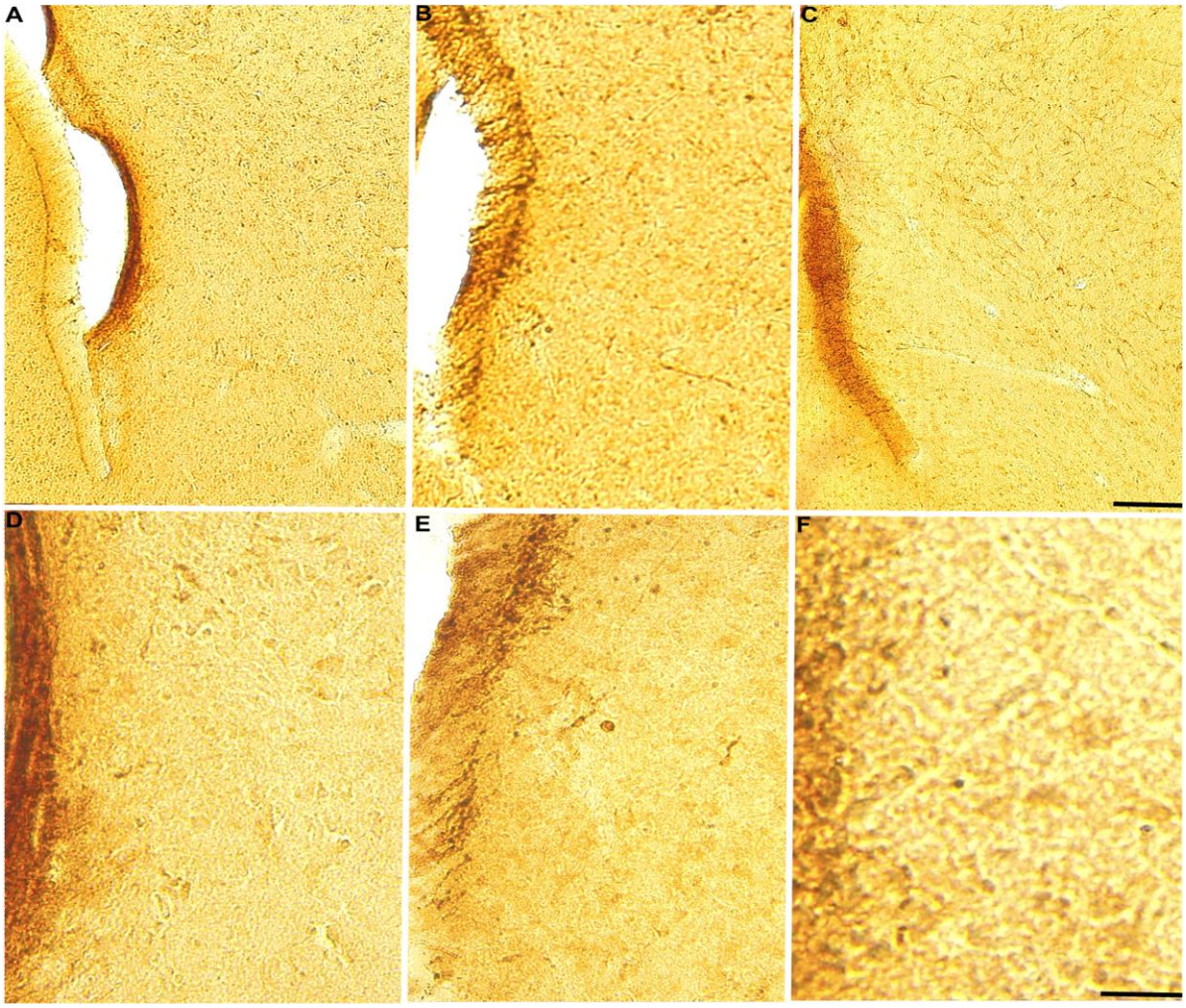


Figure 4.40: Photomicrograph of DCX immunoreactive cells in the ventricular zone juvenile (A), subadult (B) and adult (C). Scale bars in C=500um applies to A&B, Scale bars in F=50um applies to D&E. density increase to subadult but in adults. cell process increases withage

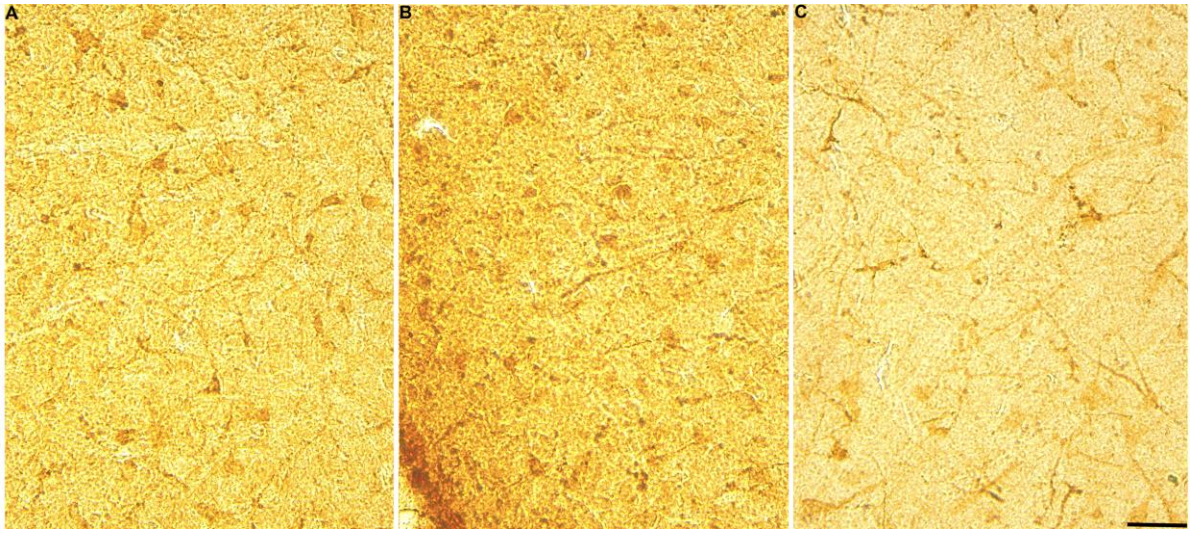


Figure 4.41: Photomicrograph of DCX immunoreactive cells in the nidopallium juvenile (A), subadult (B) and adult (C). Scale bars in C=50um applies to A, B. The density increases in the subadult but decrease in adult brain but cell processes increased in the adult brain.

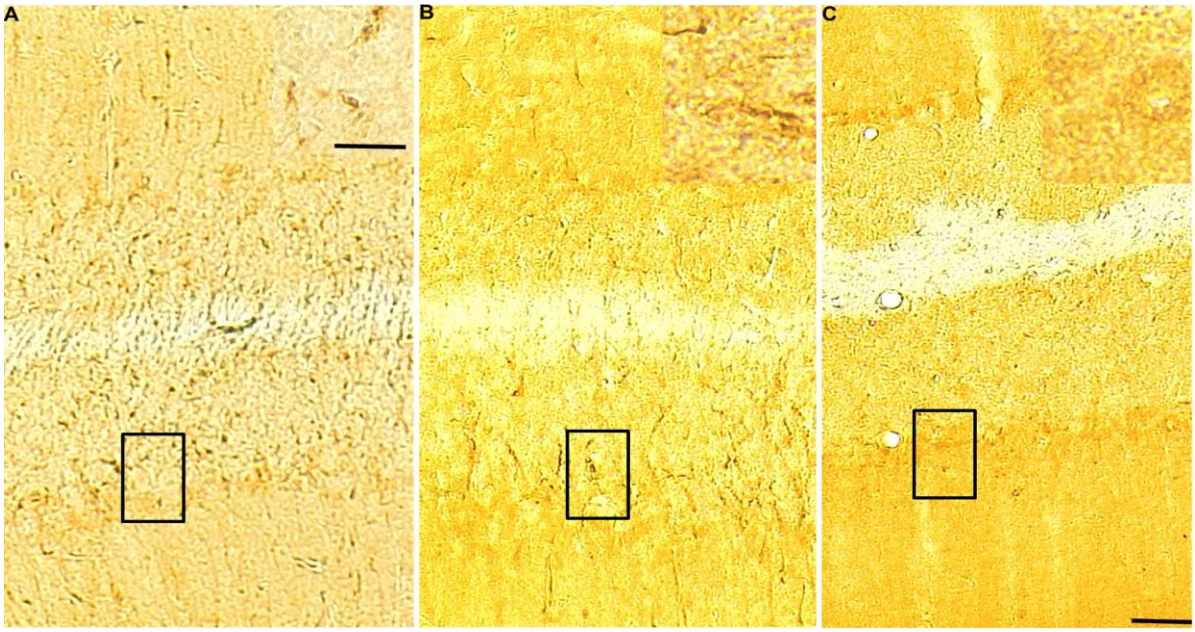


Figure 4.42: Photomicrograph of DCX immunoreactive cells in the cerebellum juvenile (A), subadult (B) and adult (C). Scale bar in C=500um applies to A&B, Scale bar in insert A=50um applies to insterts in B&C, cell density moderate in juvenile and subadult but no clear cell definitions and processes in adult

4.10 Immunofluorescence confocal immunohistochemistry

4.10.1 Qualitative analysis of neuro and gliogenesis in the Japanese quail brain

This study provides a comprehensive analysis of the cellular basis of neurogenesis and gliogenesis in the Japanese quail (*Coturnix japonica*) brain using stage- and lineage-specific biomarkers. The qualitative immunofluorescence analysis revealed that neural stem cells and progenitor populations identified by SOX2 and GFAP (Figure 4.43, 4.47) persist from the juvenile to the adult stages, and that these cells give rise to astrocytes (GFAP/S100A1) (Figure 4.44, 4.45), oligodendrocytes (GFAP/OLIG2) (Figure 4.49, 4.50) and immature neurons (GFAP/DCX) (Figure 4.44, 4.49, 4.52). The observed co-expression patterns indicate that both neurogenic and gliogenic processes remain active across developmental stages and are regionally distributed in telencephalic and mesencephalic zones. These findings confirm the persistence of neural plasticity in the quail brain and provide comparative evidence for evolutionary conservation of neurogenic mechanisms across vertebrates.

The qualitative analysis of neurogenic and gliogenic stage markers and cell types express different cell fate and cell lineage markers. The study looked at neural stem cells and progenitor cells identified by SOX2 and GFAP (Figure 4.43, 4.47, 4.52), then differentiated and matured into either glia (Figure 4.44, 4.49) or neuron identified by GFAP and S100A1 for astrocytes (Figure 4.44, 4.45), OLIG2 for oligodendrocytes (Figure 4.49, 4.50) and DCX for immature neurons (Figure 4.44, 4.49, 4.52).

Generally, SOX2, S100A1, OLIG2 and DCX co-labelling with GFAP was observed showing that stem cells actively divide and generate new stem cells, new glial cells and new neurons in the juvenile, subadult and adult quail brain (Figure 4.44, 4.49).

Thereby, the combined analysis of Sox2 and GFAP cells demonstrated co-labelling and thus indication of actively dividing stem cells (Figure 4.43- 4.54, 4.55)

These were detected at high amounts in the ventricular zones through the whole anterior–posterior axis (Figure 4.45, 4.46, 4.48, 4.52, and 4.54), hippocampus (Figure 4.45, 4.46, 4.47, 4.49), optic tectum (Figure 4.44, 4.47, 4.48, 4.53) pial surface (Figure 4.44, 4.46, 4.48, 4.52, and 4.54). The stem cells additionally have radial fibers that are GFAP positive cells (Figure 4.44). Further, small-rounded SOX2 positive cells were distributed in the juvenile brain at high densities in the hippocampal formation, the septal nuclei (SM and SL), the lateral striatum (LSt) and the nucleus taenia of the amygdala (TnA). GFAP+ and Sox2+ co-stained cells were assigned to stage 1 because they further possessed radial fibers and conclusively can be considered as active radial stem cells (Figure 4.43, 4.54).

Small-ovoidal GFAP+ and OLIG2+ were found mostly in the white mater areas including optic chiasma, optic tract, anterior and posterior commisure (Figure 4.43, 4.49). GFAP+ and OLIG2+ were also seen in the hippocampus and lining of the ventricular zones that resemble gliogenesis (oligodendrogenesis) (Figure 4.46, 4.47, 4.54).

GFAP+/ S100A1+ cells were found almost everywhere in the mesencephalon and brain stem. S100AI + positive typically resemble astro-like processes, representing astrocytes. Another type of S100AI + cell with large darkly stained nucleolus, some have processes, and some are absent (Figure 4.46, 4.47, 4.54). GFAP+ and DCX+ cells were very where in the telencephalon, mainly pallium and ventricular zones. In the ventricle GFAP+ and DCX+ were seen lining the whole length of the ventricular wall in a rostro-caudal fashion (Figure 4.46, 4.47, 4.49).

GFAP **S100A1** **DAPI** **MERGED**

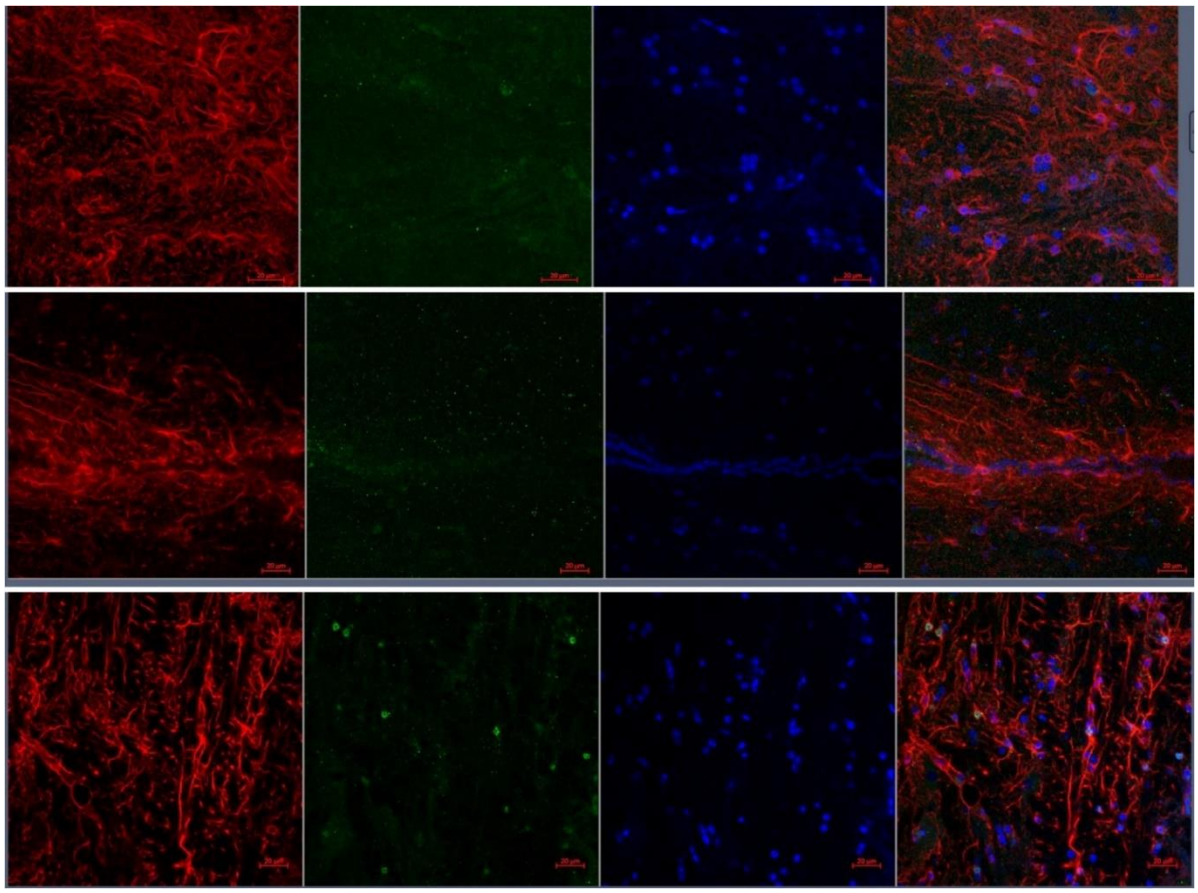


Figure 4.43: Photomicrograph of GFAP/SOX2 immunofluorescence the septum, ventricle and optic tectum in the juvenile, subadult and adult quail brain. Colocalization was observed in the optic tectum. GFAP indicated as red, SOX2 as green and DAPI: blue. Scale Bar: 20μm

GFAP **S100A1** **MERGED**

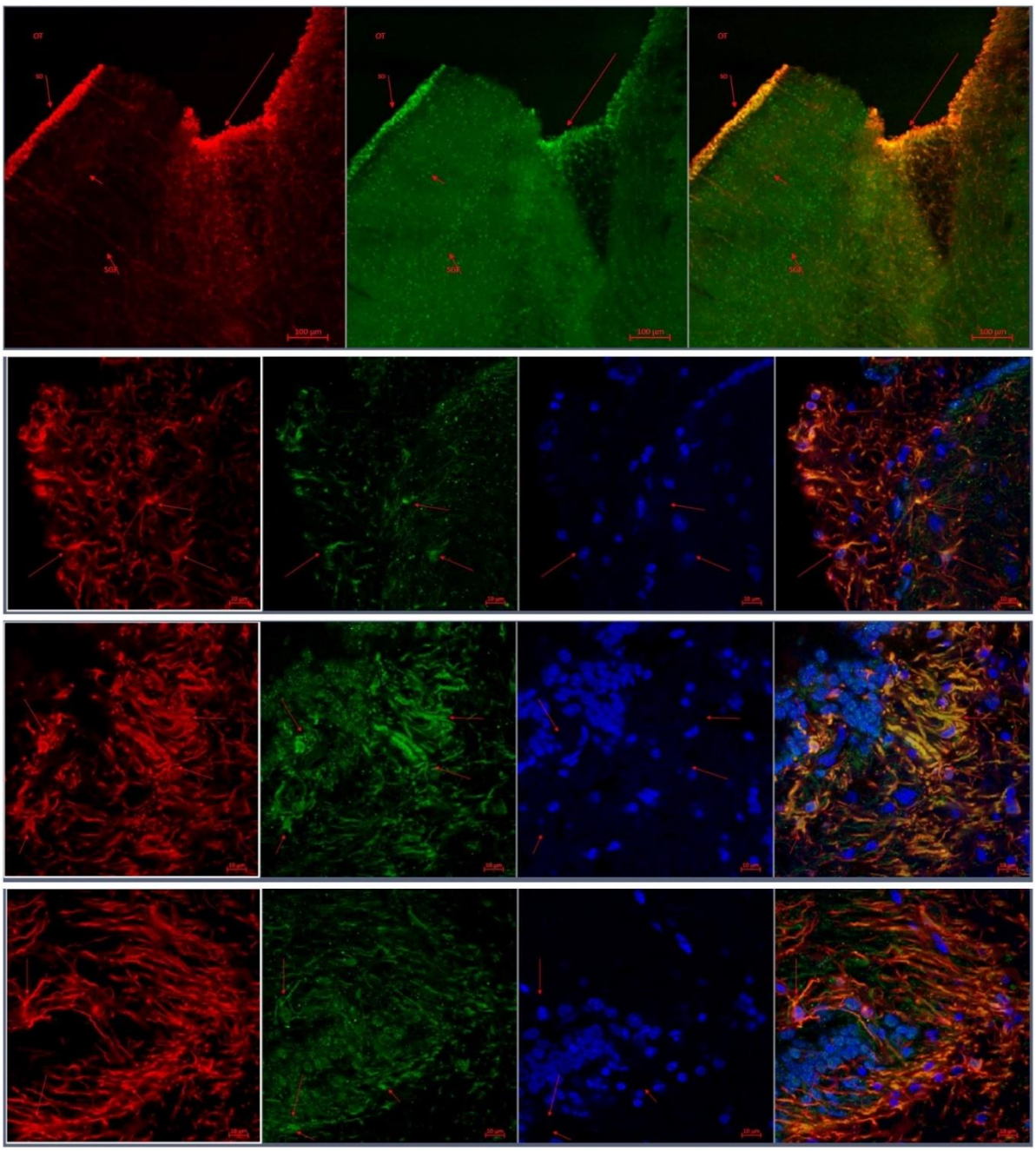


Figure 4.44: Photomicrograph; panoramic view of GFAP and S100A1 in the optic tectum edge showed an intense colocalization in the SO and SGF without dapi. Higher magnification of optic tectum in juvenile, subadult and quail brain. GFAP: red, S100A1:green and DAPI: blue. Scale Bar: 100µm, 20µm

GFAP **SOX2** **MERGED**

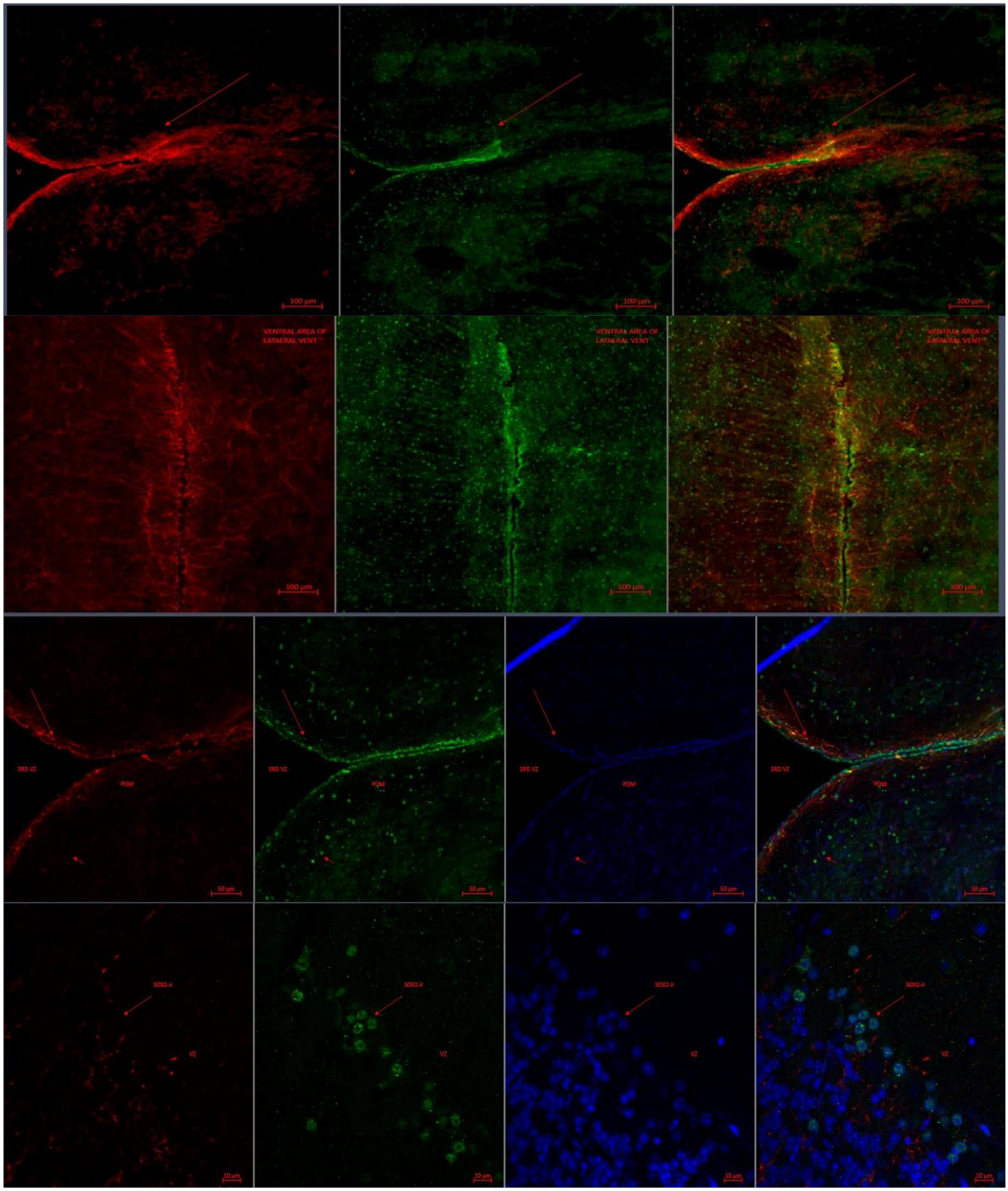


Figure 4.45: Photomicrograph: panoramic view of colocalization of GFAP and SOX2 immunofluorescence in the ventricular zone, the lateral and 3rd ventricle in juvenile, subadult and adult quail brain. Colocalization is more intense in the juvenile and subadult ventricle. GFAP: Red, SOX2: green, DAPI: blue. Scale Bar: 100µm, 20µm

GFAP **S100A1** **DAPI** **MERGED**

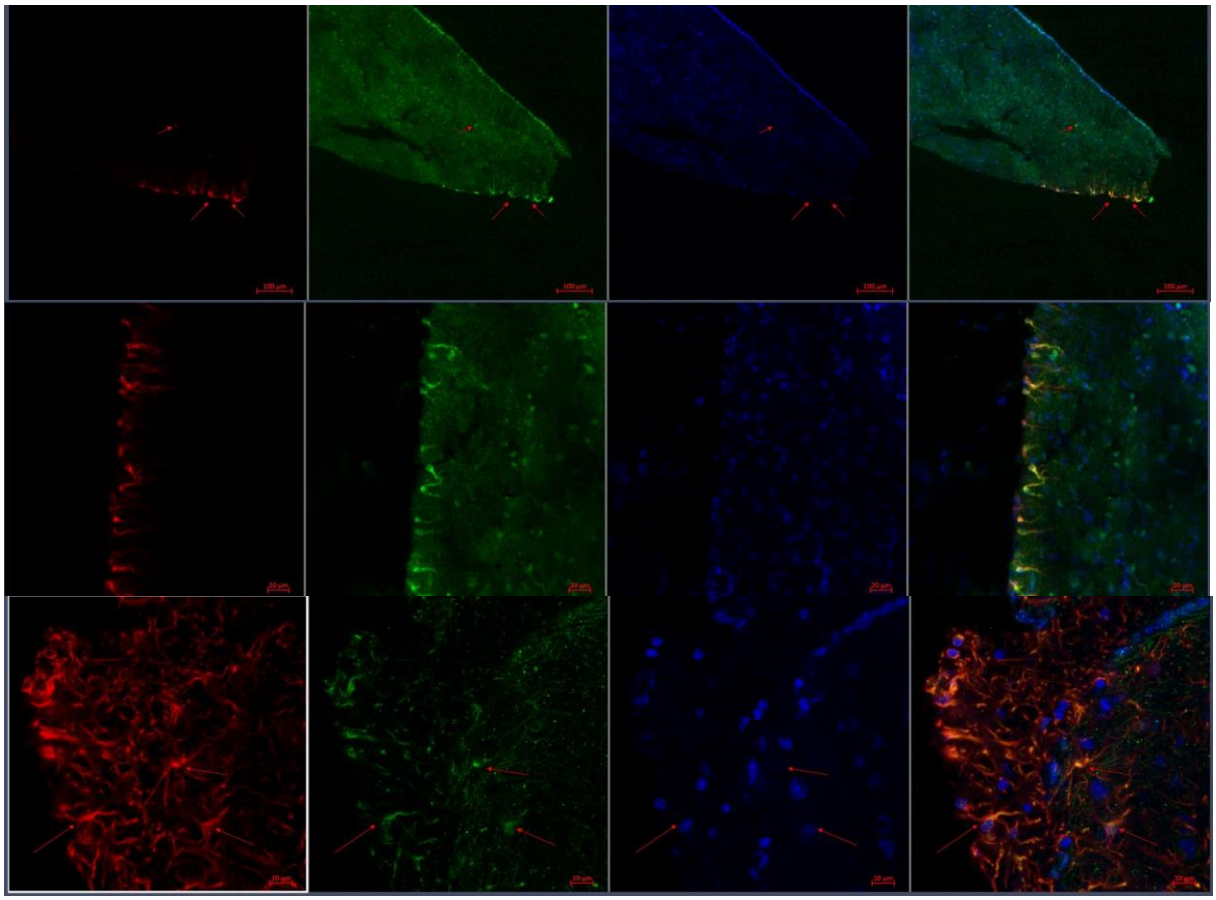


Figure 4.46: Photomicrograph of colocalization of GFAP and SOX2 immunofluorescence in in the hippocampus of juvenile, subadult quail brain. Intense colocalization is seen at the V-area and hippocampal pial surface. Higher magnification showed the core area of the hippocampus both were positive for GFAP and SOX2. GFAP: Red, SOX2: green, DAPI: blue. Scale Bar: 100µm, 20µm

GFAP **SOX2** **MERGED**

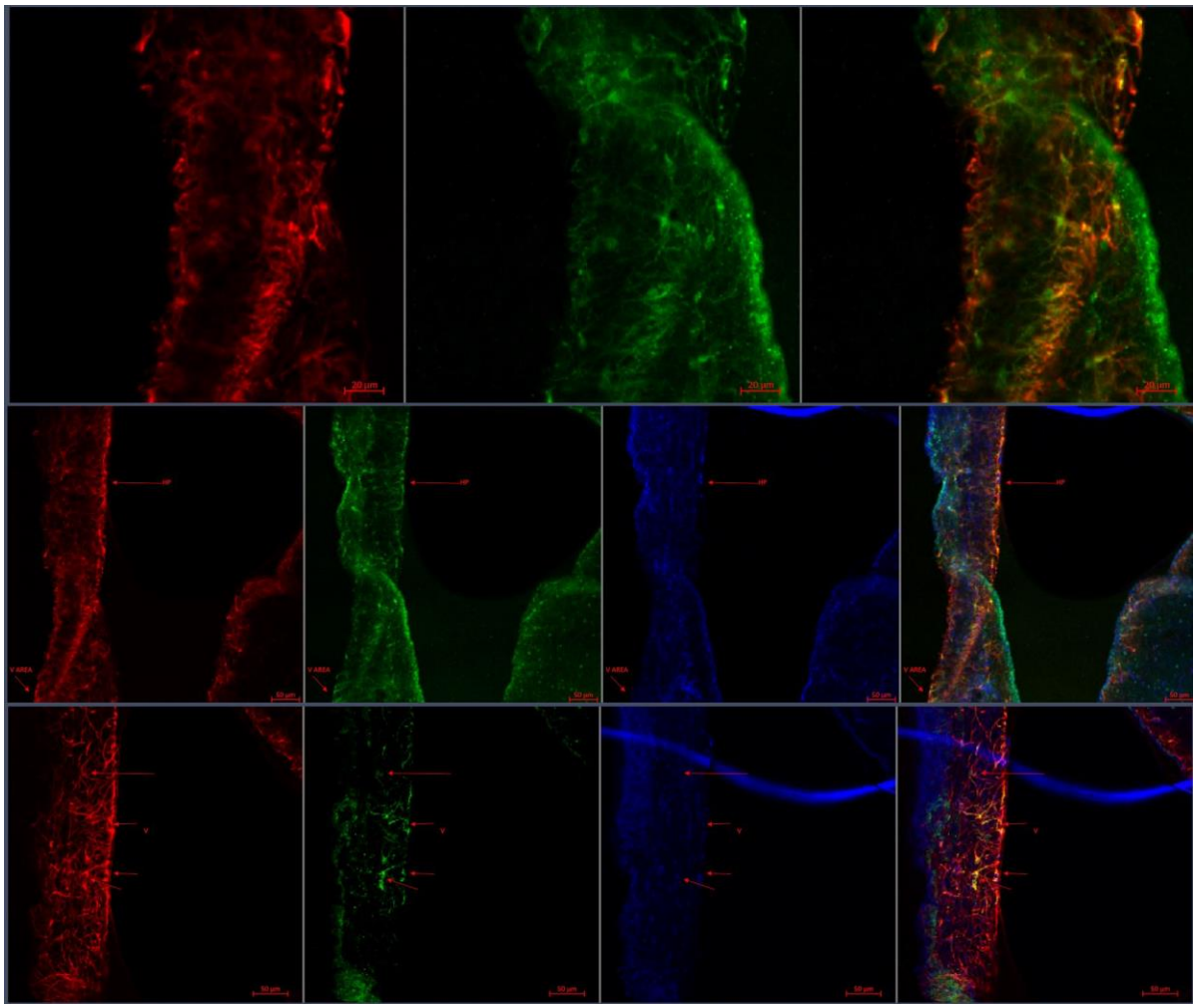


Figure 4.47: Photomicrograph of colocalization of GFAP and SOX2 immunofluorescence in in the hippocampus of juvenile quail brain. Intense colocalization is seen throughout the hippocampus. GFAP: Red, SOX2: green, DAPI: blue. Scale Bar: 100µm, 20µm

GFAP SOX2 DAPI MERGED

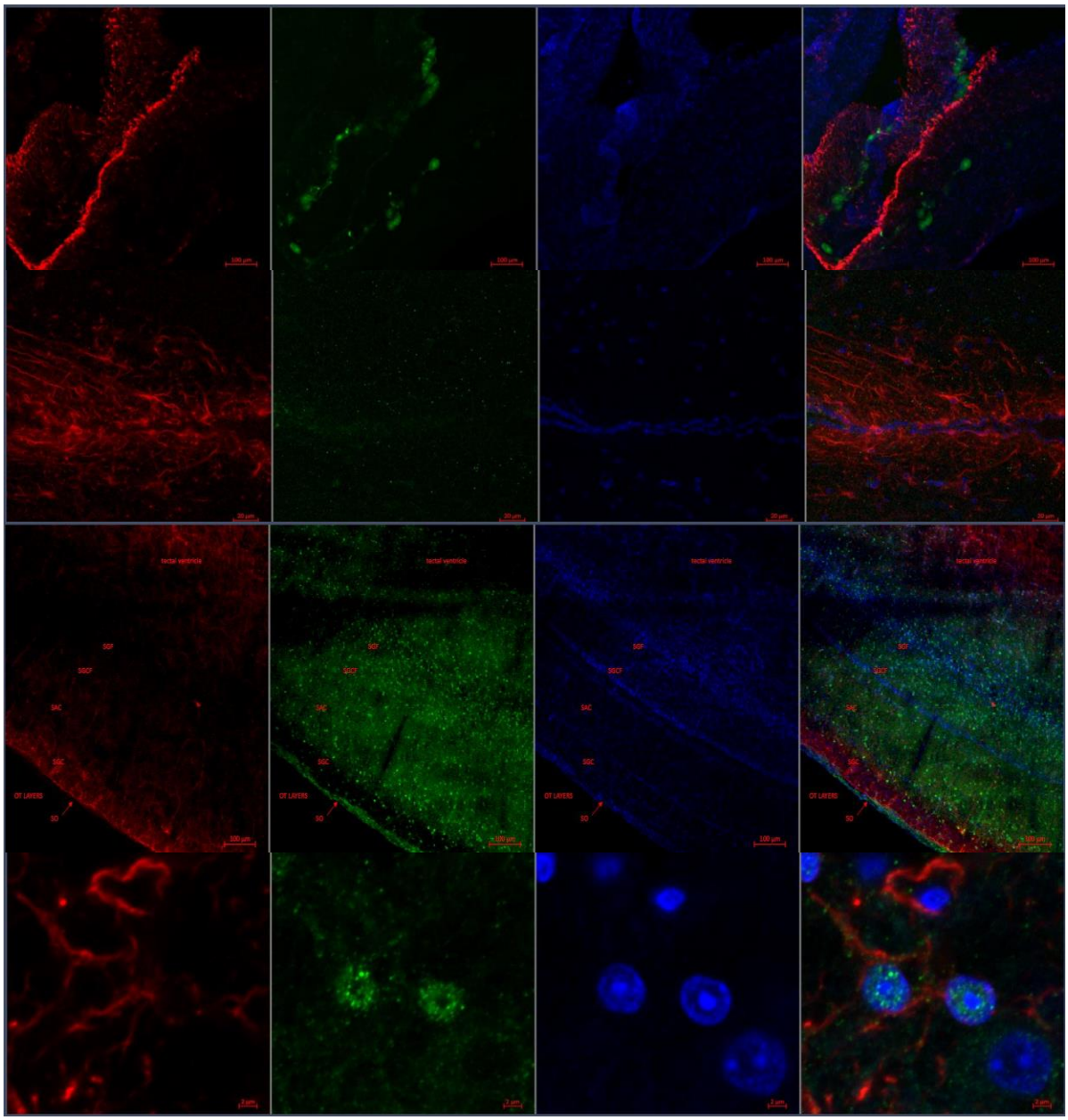


Figure 4.48: Photomicrograph of GFAP and SOX2 immunofluorescence in the ventricular zone and optic tectum of juvenile and subadult quail brain. Higher magnification showed low intensity of colocalization at the SO and SGF. GFAP: red, SOX2: green and DAPI: blue. Scale Bar: 100µm, 20µm

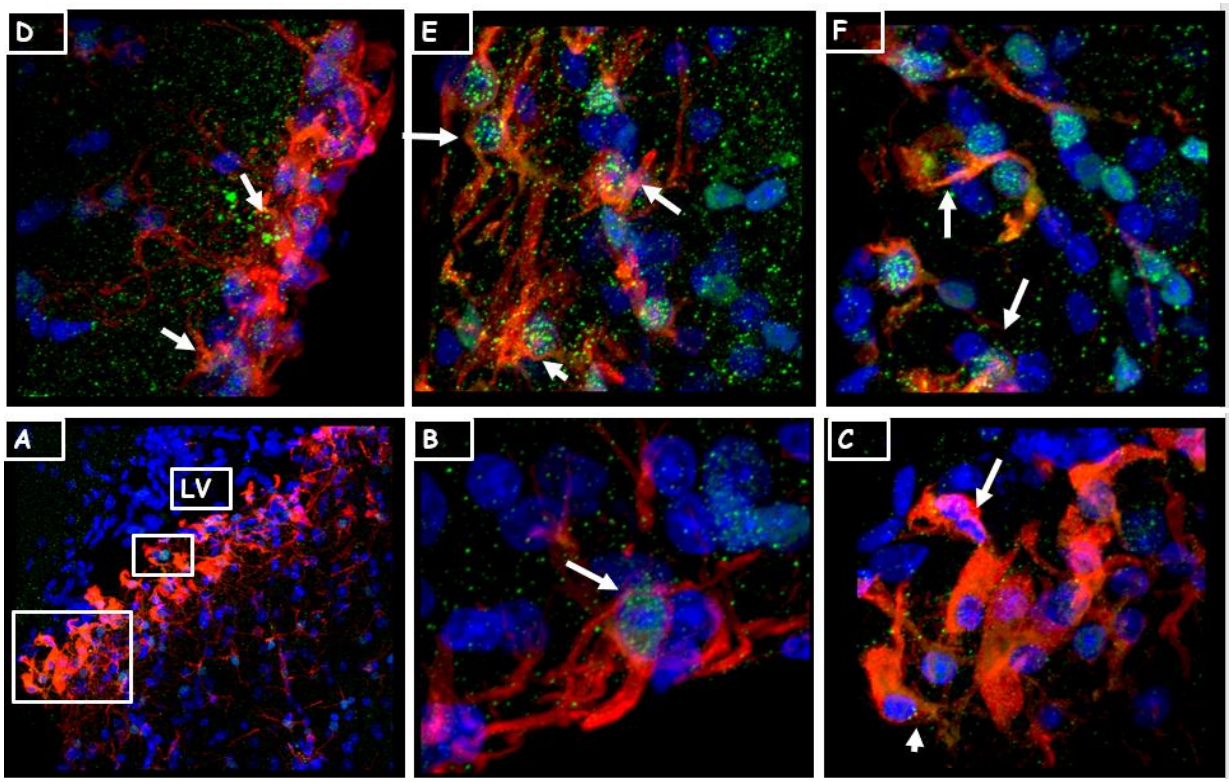


Figure 4.49: Photomicrograph of GFAP and SOX2 immunofluorescence in the lateral ventricle in the quail brain. Both GFAP and SOX2 where positive along the ventricular axis. Higher magnification showed areas of high intensity of colocalization. GFAP: red, SOX2: green and DAPI: blue. Scale Bar:100µm, 20µm

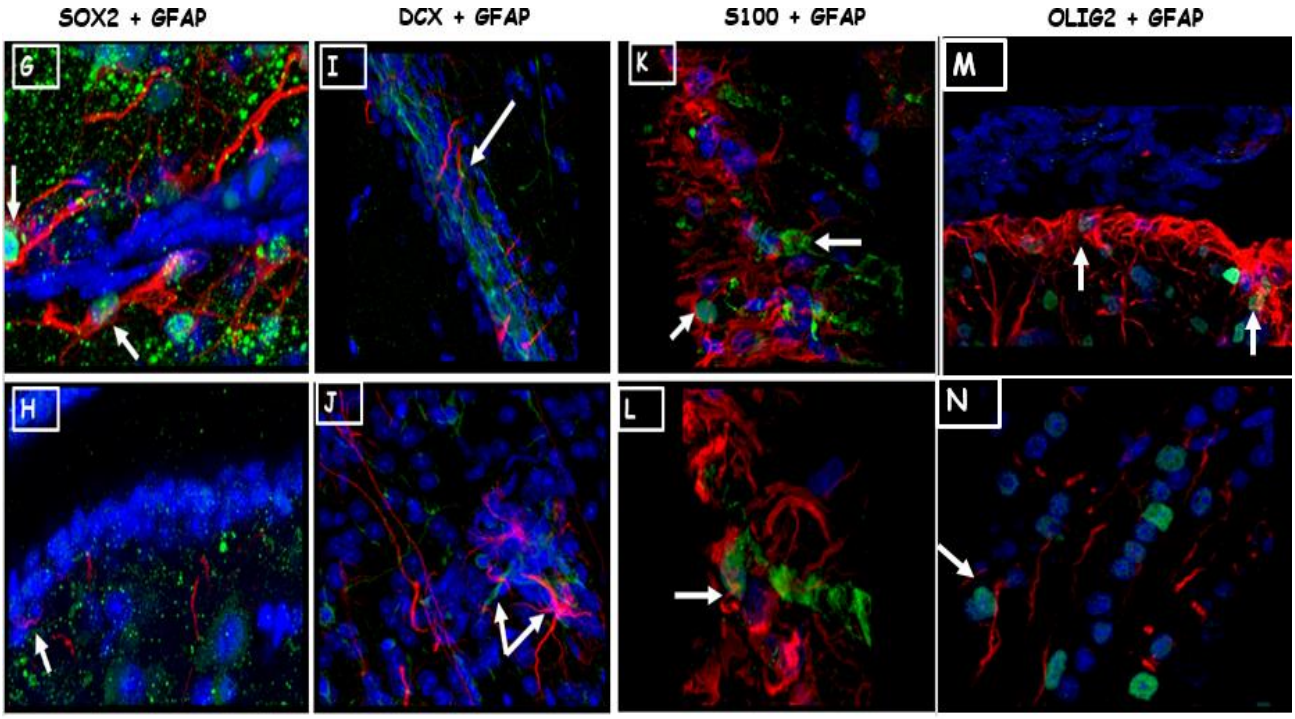


Figure 4.50: Double labelling immunohistochemistry in the ventricular zone by SOX2/GFAP **(G, H).** Lateral ventricle and pallium **(I, J)** showed colocalization of immature neurons DCX and GFAP. GFAP and S100A1 in the pial surface showed high intensity **(K, L).** GFAP and OLIG2 in the ventricular wall **(M)** and optic chiasma **(N)** showed oligodendrocytes arranged in rows. Scale bar: G,H,J, K, L, M, N= 20µm

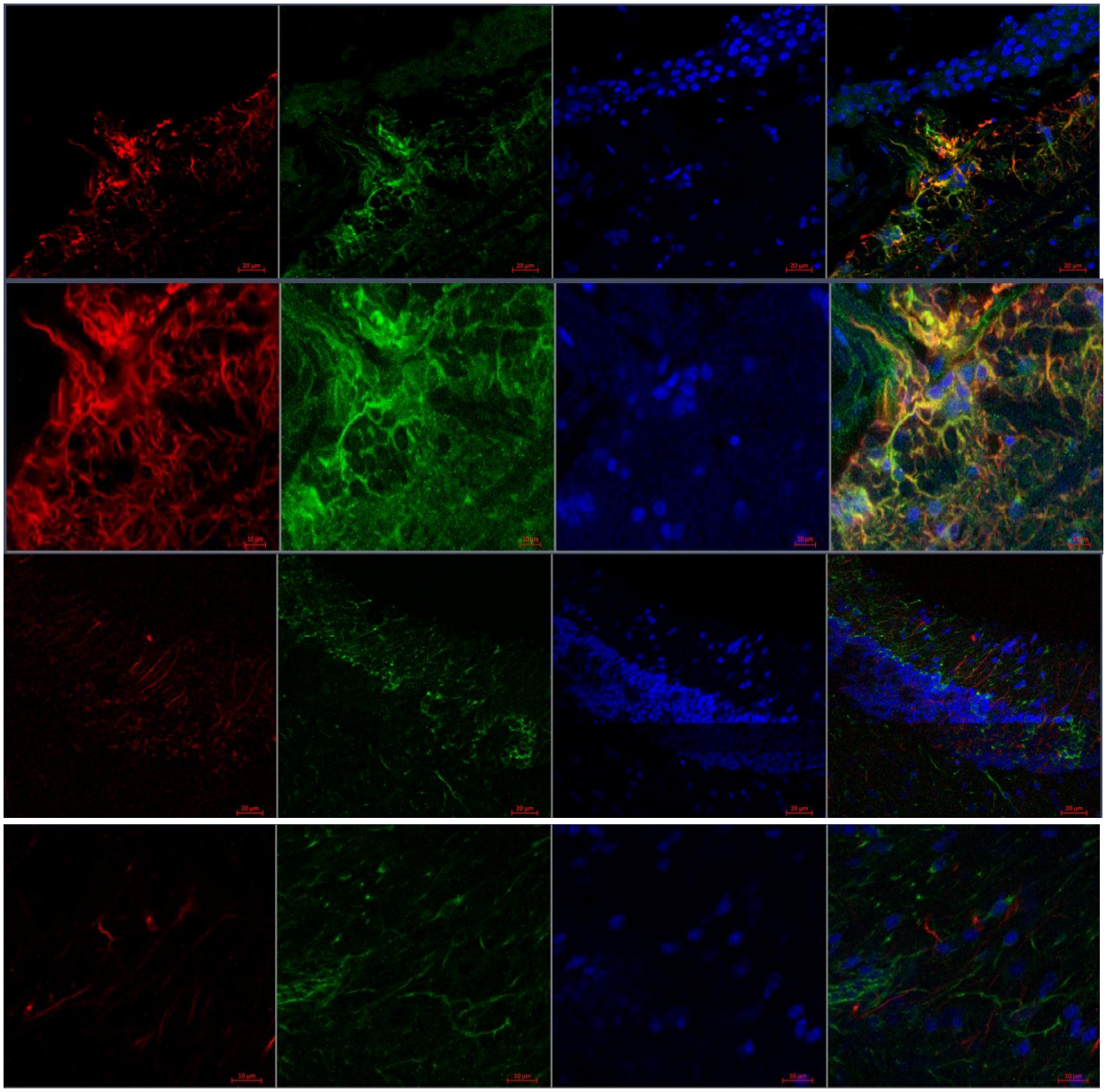


Figure 4.51: Photomicrograph of GFAP and DCX immunofluorescence in the ventricular zone of juvenile quail brain showing intense colocalization along the ventricular wall axis. Higher magnification showed colocalization. GFAP: red, DCX: green and DAPI: blue. Scale Bar: 100µm, 20µm

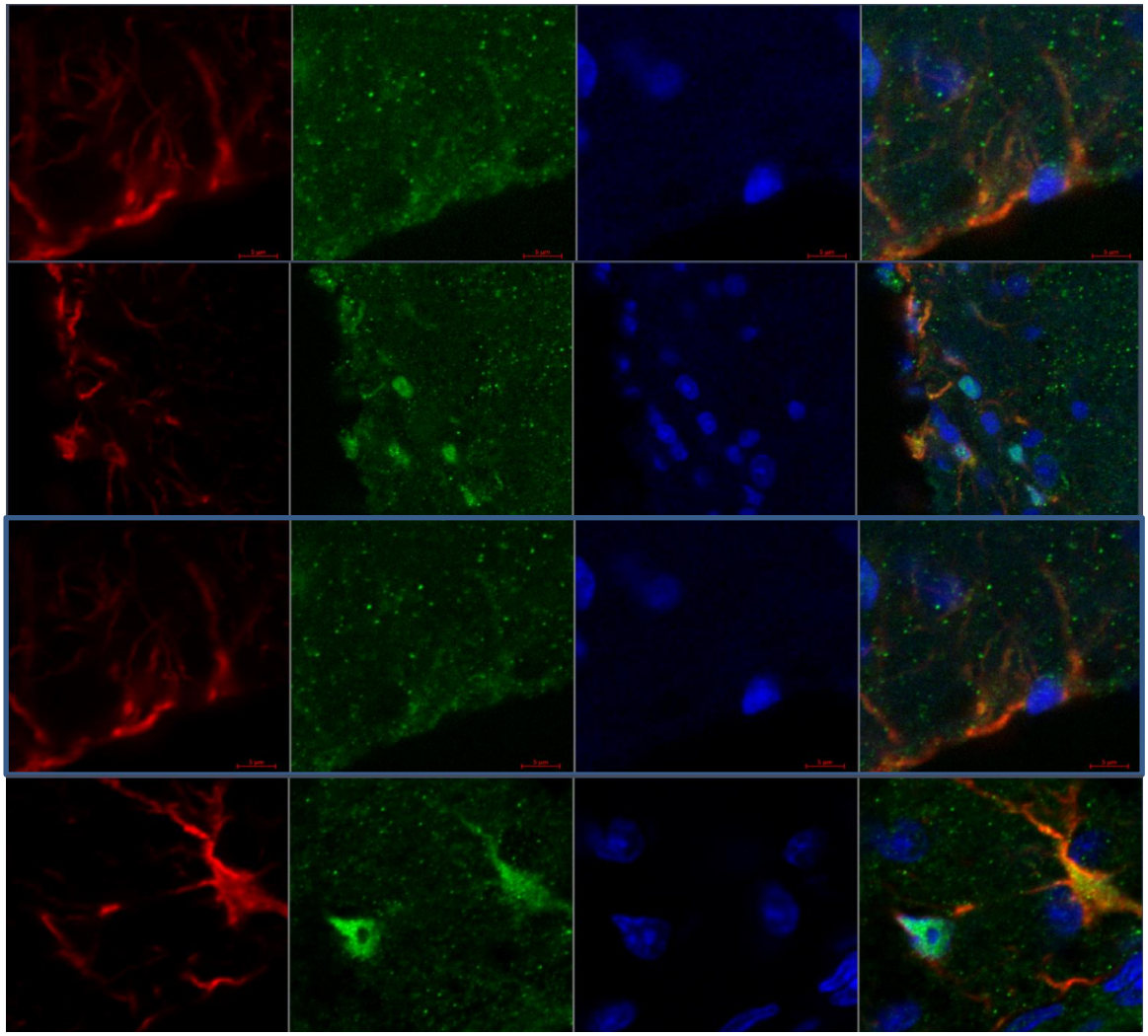


Figure 4.52: Double labelling immunohistochemistry of GFAP/SOX2 and GFAP/DCX in the Juvenile quail brain showing the pial surface, optic tectum and cerebellum. GFAP: red, SOX2/ DCX: green and DAPI: blue. Scale Bar: 100µm, 20µm

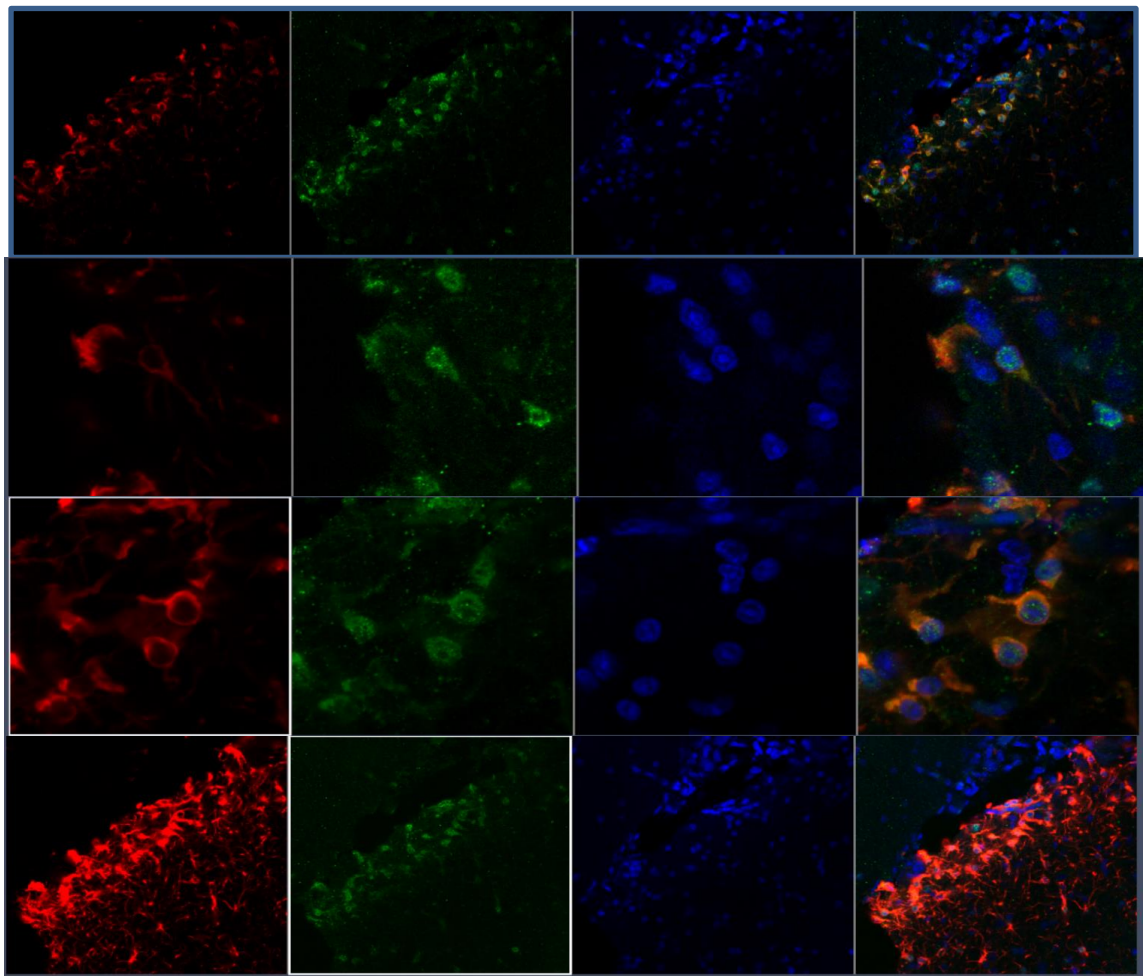


Figure 4.53: Double labelling immunohistochemistry of GFAP/SOX2 and GFAP/OLIG2 in the quail brain showing the ventricular wall area of colocalization. GFAP: red, SOX2/ OLIG2: green and DAPI: blue. Scale Bar: 100µm, 20µm

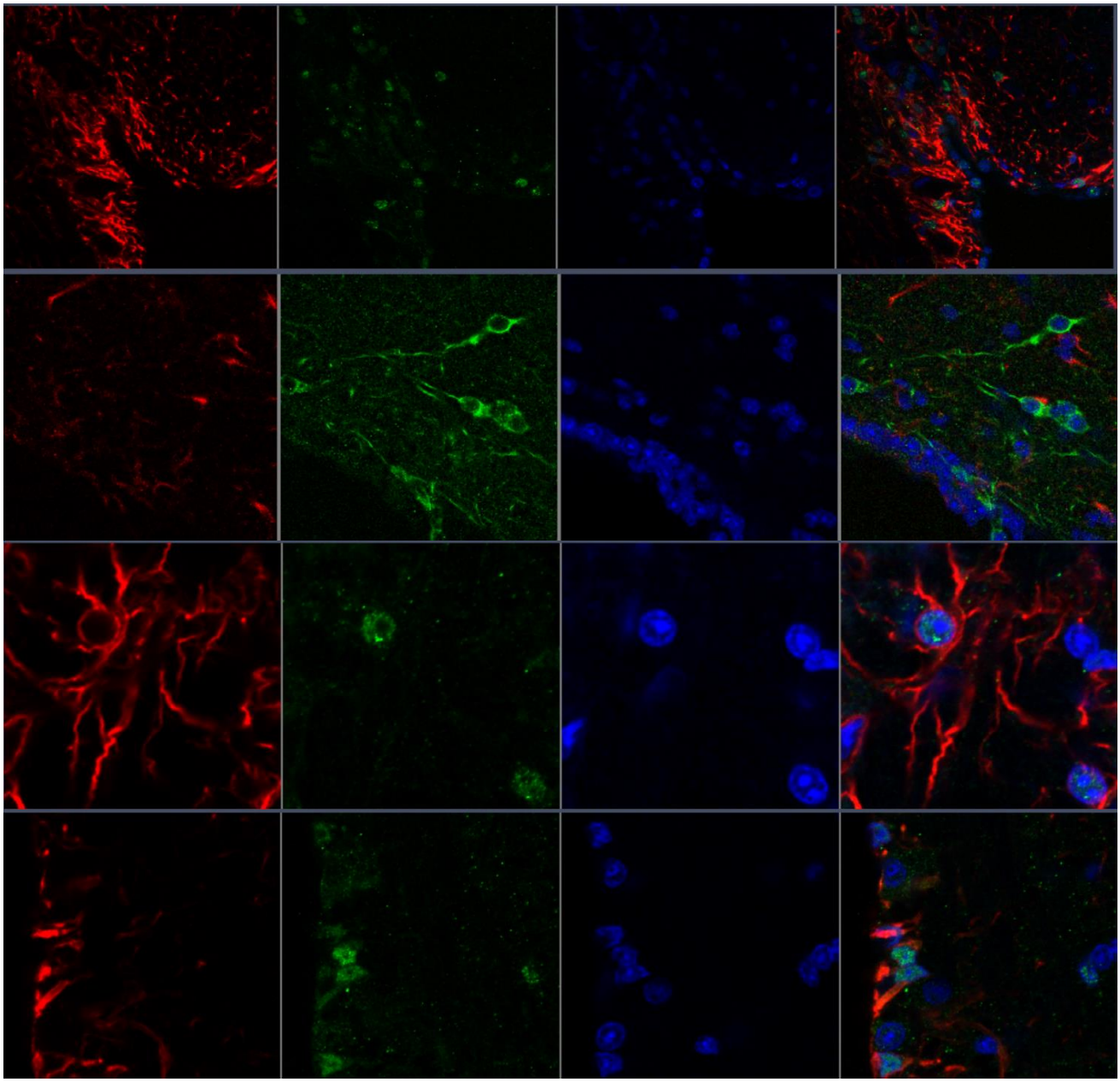


Figure 4.54: Double labelling immunohistochemistry of GFAP & S100A1 in the Juvenile quail brain in the mesencephalon. GFAP: red, S100A1: green and DAPI: blue. Scale Bar: 100µm, 20µm

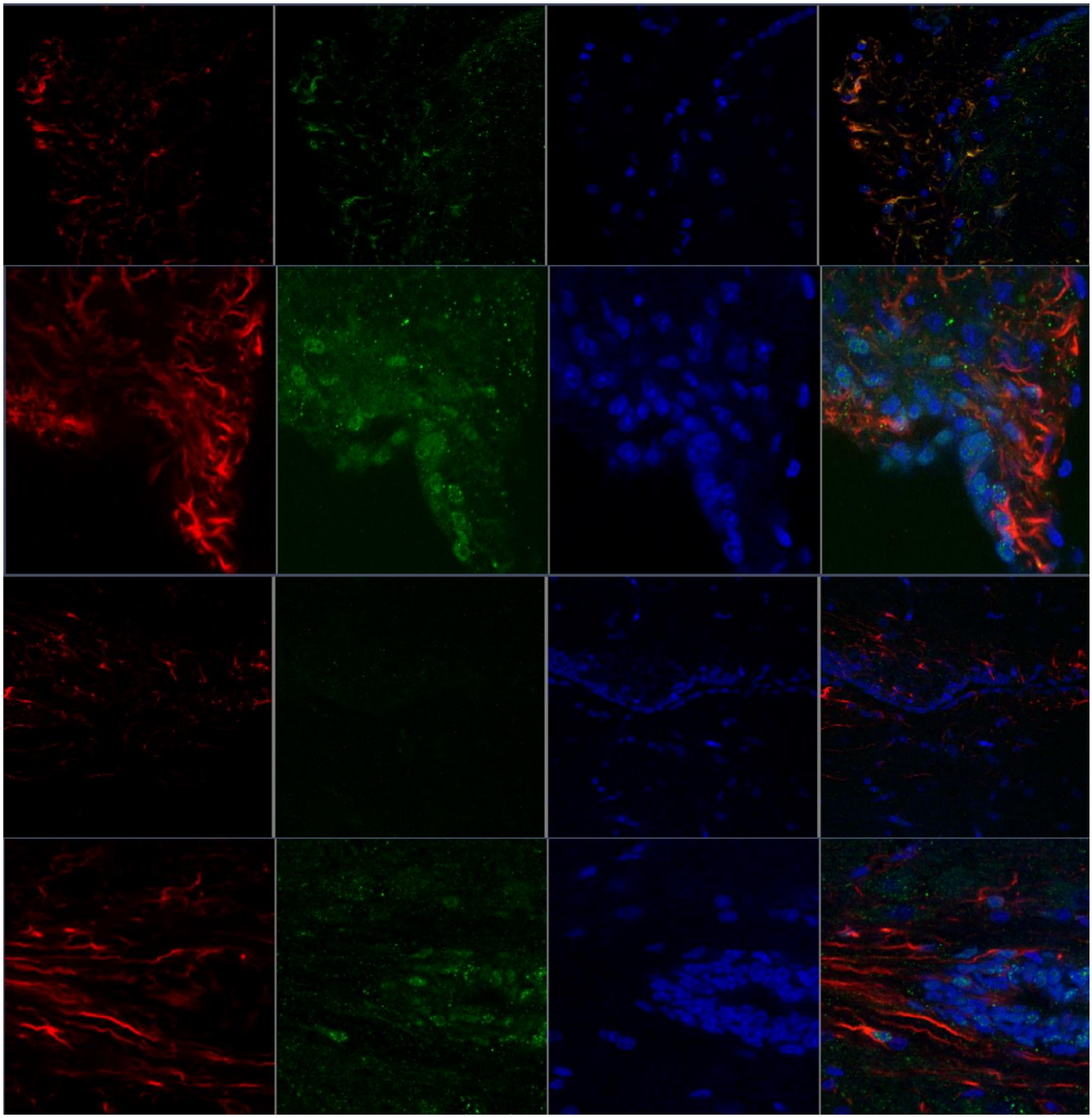


Figure 4.55: Double labelling immunohistochemistry of SOX2 & GFAP in the subadult quail brain showing area of optic tectum and 3rd ventricle. GFAP: red, SOX2: green and DAPI: blue. Scale Bar: 100µm, 20µm

4.11 Ultrastructural identification of glial, neuronal cell and mitochondria in the male Japanese quail brain by transmission electron microscopy (TEM)

This chapter aims to elucidate the structure and localization of glial cells, neurons, and mitochondria in specific brain regions of the Japanese quail. The findings of this study concentrate on the pallium, mesencephalon, optic tectum, and cerebellum in juvenile, subadult, and adult male Japanese quail brains. The study utilized the methodologies outlined by Garcia-Cabezas *et al.* (2016) and Panzica *et al.* (1994) to identify the cellular components.

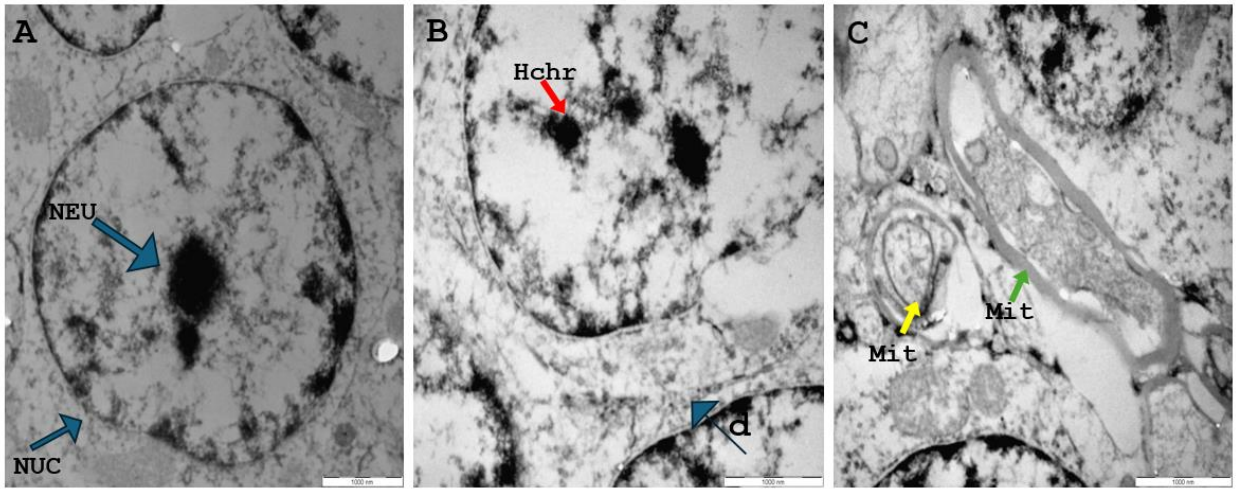


Figure 4.56: Ultrastructural photomicrography of neuron, glia and mitochondria in the cerebellum in juvenile, subadult and adult quail brain (A-C). Blue arrow showed nucleus and nucleolus (A-C): neuron nucleus with dark nucleolus, yellow arrow is round mitochondria, red arrow is heterochromatin, and green arrow is elongated mitochondria. Scale bar = 1000nm

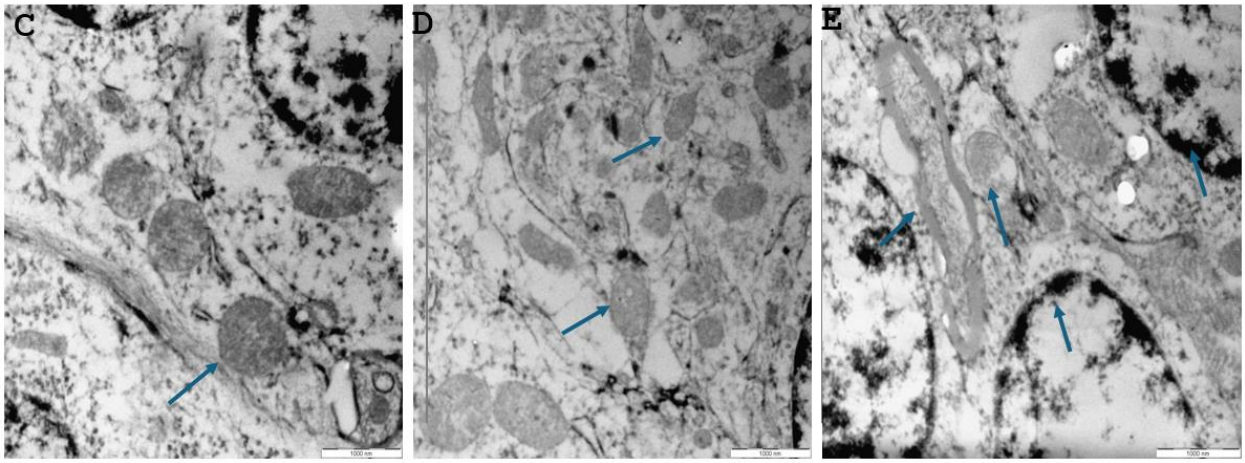


Figure 4.57: Ultrastructural photomicrography of cerebellum in the juvenile, subadult, adult quail brain with abundant microglia which has a darkly stained body (D). Heterochromatin and nissl bodies (A-C), elongated and round mitochondria are seen scattered in the cerebellum (C-E) Scale bar = 1000nm

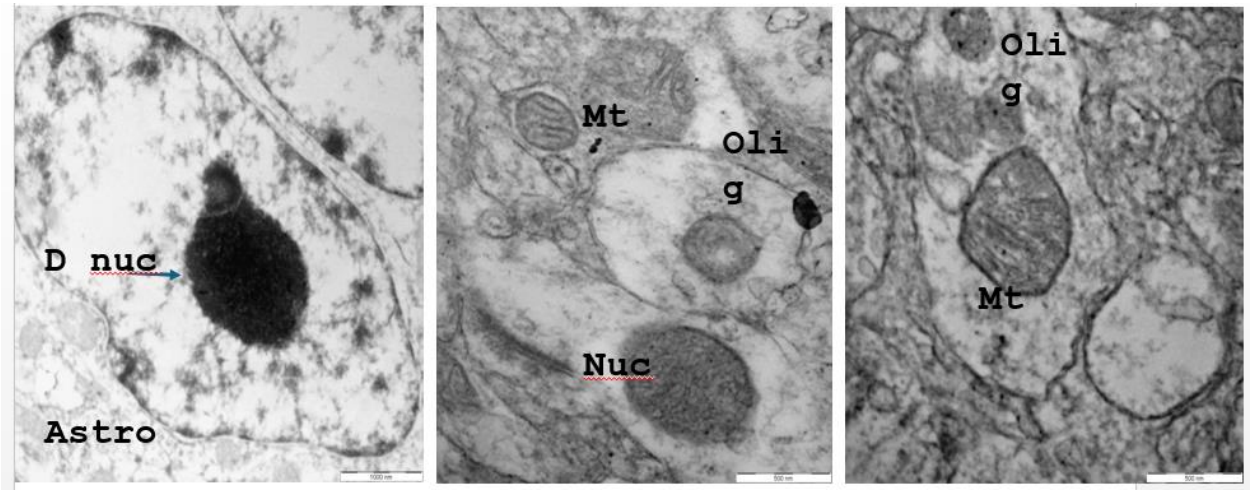


Figure 4.58: Ultrastructural photomicrography of glia and mitochondria in the juvenile, subadult and adult quail brain. Heterochromatin aggregation and granules in the nucleus, . astrocytes (Astro) **(A)**. In (B) showed dendrite surrounded by the nissl bodies with it nucleus, oligodendrocyte (Olig) and round mitochondria. (C) showed the presence of rounded mitochondria with a typical cristae (mt) and oligodendrocyte (Olig). Scale bar : A=1000nm, B-C=500nm

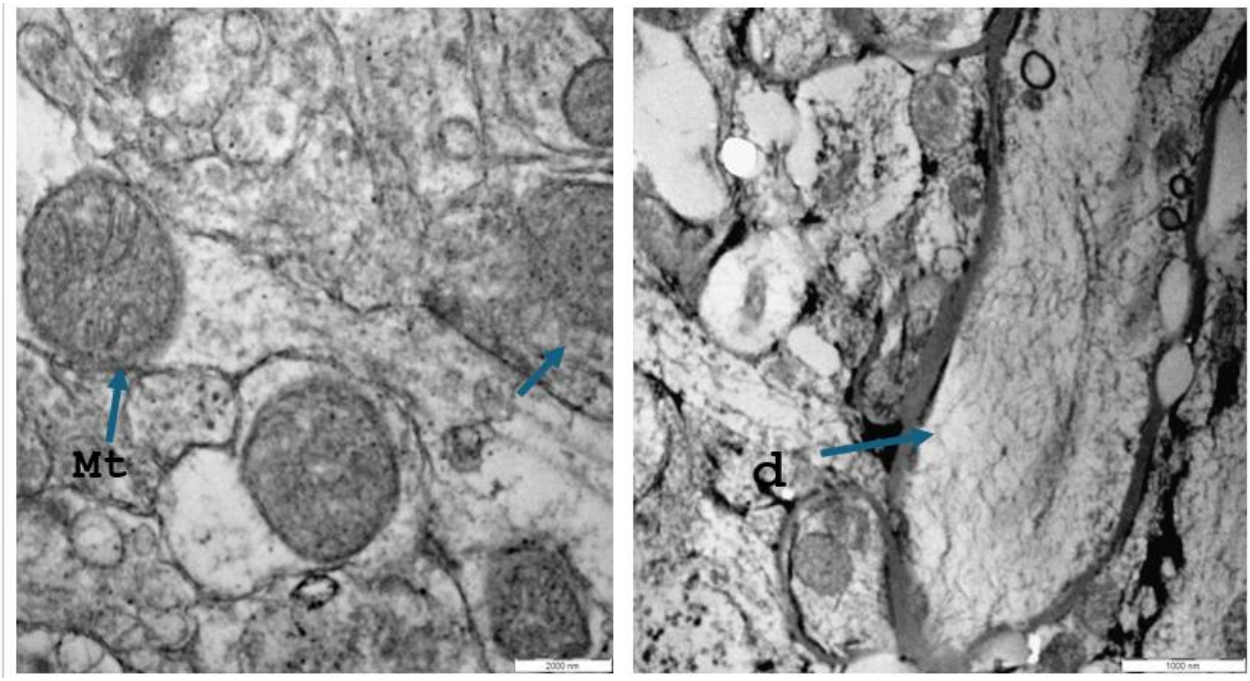


Figure 4.59: Ultrastructural photomicrography of neuron and mitochondria in juvenile and subadult quail brain in the pallium. (A) shoed round mitochondria in blue arrow and oligodendrocyte. (B) showed heterochromatin, dendrite (d) with blue arrow surrounded by the nissl bodies, small round mitochondria. Scale bar, A=2000nm, B=1000nm)

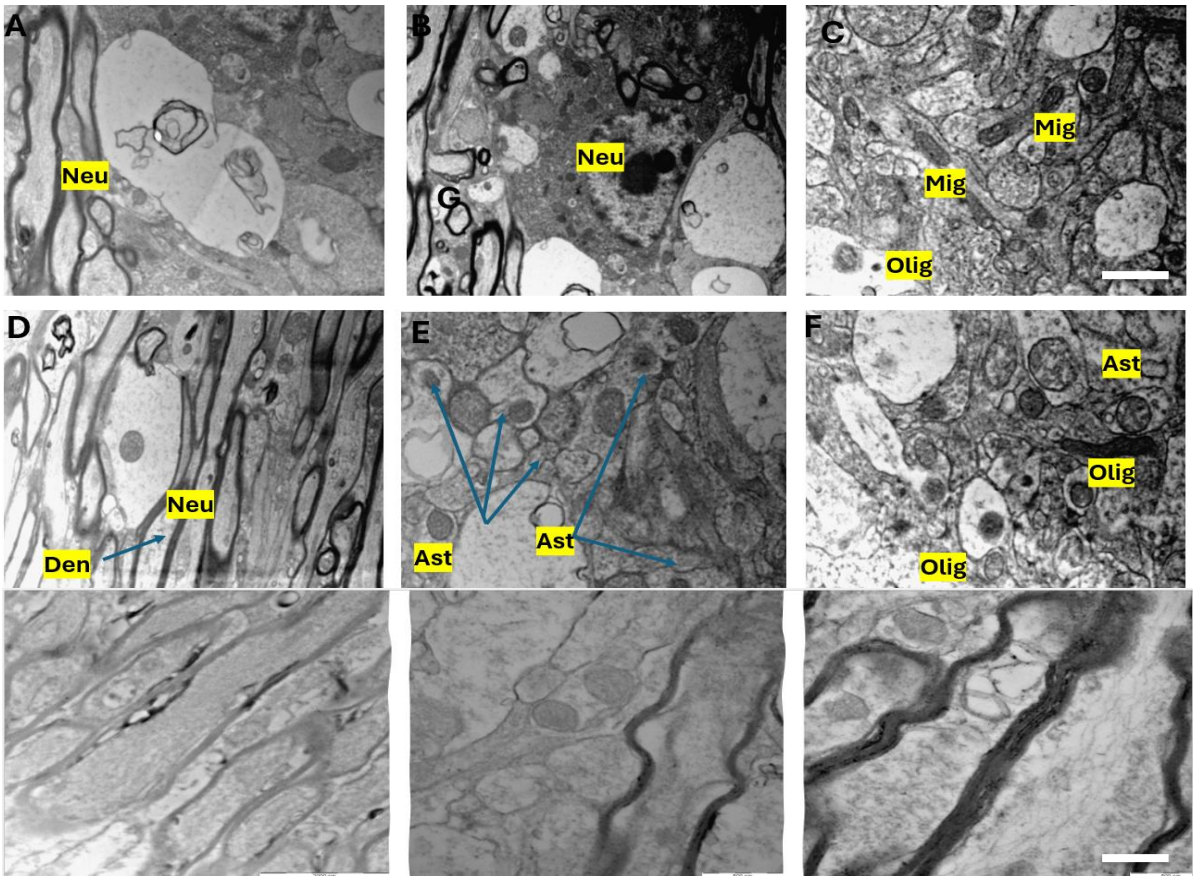


Figure 4.60: Ultrastructural photomicrography of the optic tectum in the juvenile (A-C), subadult (D-F) and adult brain (G-I). The optic tectum in the juvenile showed more neurons with its dendrite (A-B), then oligodendrocyte, mitochondria and heterochromatin granules (C). The subadult showed neuron and dendrites (D), astrocytes, oligodendrocytes and microglia (E), oligodendrocytes, astrocytes and mitochondria (F). The adult brain showed dendrites of neuron, round mitochondria (G-I). Ast, astrocytes, Mit: mitochondria, Olig: oligodendrocytes and Mig: microglia. Scale bar: A-F = 500nm, G-1= 1000nm.

CHAPTER FIVE: Discussion

In this chapter, we discuss neural and glial development, maturation, and potential regenerative capacities by mapping their expression across various brain regions at different developmental stages.

5.1 Distribution and characterization of SOX2 immunoreactive cells in the quail brain.

In the telencephalon, the presence of SOX2-ir within the developing or adult nervous system signifies the presence of stem cells (Stevanovic *et al.,* 2021). Consequently, its moderate distribution across Hyperpallium, Mesopallium, and Nidopallium elucidates the diverse roles and levels of neurogenic and gliogenic activity at various stages of brain development. In juvenile specimens, this may indicate active neurogenesis or gliogenesis, as traditionally understood (Bertolini *et al.,* 2016; Stevanovic *et al.,* 2021). This process is critical for the development of sensory processing, cognitive functions, and complex behaviors, which are functions of the pallium (Güntürkün *et al.,* 2021). Similarly, during the subadult stage, the high density observed in the mesopallium may suggest structural and functional maturation. The observed decline in adulthood across these regions may be attributed to a reduction in neurogenic activity, consistent with previous studies (Nkomozepi, Mazengenya, and Ihunwo, 2018), which reported a decline in the expression of neurogenesis markers with age in the developing quail brain. Furthermore, the presence of SOX2-ir cells in the adult stage indicates an elevated level of gliogenesis, which is essential for maintaining and protecting neurons by providing support, insulation, and homeostasis (Arai and Lo, 2017).

This pattern aligns with the brain's developmental requirements, emphasizing growth and adaptation in earlier stages, and stability and maintenance in adulthood (Arai and Lo, 2017), becoming increasingly significant in older quails to sustain and protect established neural structures. Notably, the similar expression patterns of SOX2 and PCNA in the optic bulb, pallium, and subpallium suggest a high level of cellular activity, corroborating the observations' inclination towards gliogenesis rather than neurogenesis, as cell proliferation is more prevalent in gliogenesis.

The high density of cells observed in the ventricular zones (VZ) and subventricular zones (SVZ) of juveniles and subadults, particularly along the rostrocaudal axis and more concentrated in the caudal regions, suggests significant neurogenic or gliogenic activity crucial for brain development and the formation of neural circuits. In adult quails, the moderate density of these cells may reflect the anticipated reduction in neurogenesis associated with maturity. However, the presence of some SOX2-immunoreactive (SOX2-ir) cells in the subpallial areas implies that, although neurogenesis diminishes with age, gliogenesis continues to play a role in supporting and maintaining established neural structures (Arai and Lo, 2017).

In juvenile and subadult quails, the presence of SOX2-immunoreactive (SOX2-ir) cells in the septum, TSM, nucleus posterioris amygdalopalli, and nucleus accumbens indicates active neurogenesis and gliogenesis, which supports the development of regions associated with emotion, reward processing, and social behaviors (Gutierrez-Ibanez *et al.,* 2016; Nakamori *et al.,* 2019). The moderate density observed in areas such as the fasciculus prosencephala lateralis, ventral tegmental area, TnA, and globus pallidus likely reflects ongoing maturation and functional integration of these regions.

In adult quails, the high density of SOX2-ir cells in the lateral septum and nucleus accumbens suggests that gliogenesis plays a crucial role in maintaining and protecting the established neural structures involved in reward and emotional processing (Hikida *et al.,* 2016). Conversely, the low density in other subpallial structures, including the medial septum, may indicate a transition from neurogenesis to gliogenesis, aligning with the brain's focus on stabilizing and supporting existing neural networks rather than forming new ones (Zhao *et al.,* 2006). This pattern is consistent with the known decrease in neurogenic activity and the increased importance of glial support in the mature brain (Zhao, Chai, and Frotscher, 2006; Nkomozepi, Mazengenya, and Ihunwo, 2019).

The high density of SOX2-ir cells in the medial and lateral habenula of juvenile quail brains suggests robust neurogenic and gliogenic activity, which is critical for the development of neural circuits involved in emotional and motivational processing (Ables *et al.,* 2023). As quails mature into subadults and adults, the density of SOX2-ir cells decreases to moderate and low levels, respectively, indicating a reduction in the need for neurogenesis as the brain shifts its focus towards maintaining and supporting established neural networks. This pattern aligns with the broader trend observed in other brain regions, where early developmental stages are characterized by high levels of neurogenic activity, which taper off as the brain reaches maturity and prioritizes the maintenance of existing neural structures (Owji and Shoja, 2020).

In the hippocampus, the distribution of SOX2-ir cells in juvenile and subadult quail brains reveals distinct regional patterns that may reflect differential levels of neurogenic activity and progenitor cell proliferation within this brain structure (Bueno and Martínez, 2021).

The high density of SOX2-ir cells in the ventral area of the hippocampus suggests a robust population of neural progenitor cells localized within this region during early postnatal development. The ventral hippocampus is known to be involved in various cognitive and emotional processes, including stress regulation and spatial memory (Gulyaeva, 2015; Surget and Belzung, 2022). The presence of a high density of SOX2-ir cells in this region may indicate ongoing neurogenesis and cellular turnover associated with these functions.

In contrast, the dorsal region of the hippocampus, encompassing layer II and its dorsal extent, demonstrated a moderate density of SOX2-ir cells in juvenile and subadult quail brains. This pattern indicates a lower level of neurogenic activity compared to the ventral hippocampus, yet it still signifies the presence of proliferating neural progenitor cells within these regions. The dorsal hippocampus is primarily associated with spatial navigation and memory processing (Gulyaeva, 2015; Eichenbaum, 2017; Sodoma *et al.,* 2021; Surget and Belzung, 2022), and the observed SOX2 expression may reflect the maintenance and plasticity of neuronal circuits involved in these functions. A moderate density of SOX2-ir cells was also observed in the area parahippocampalis (APH), dorsolateral cortical area (CDL), and piriform cortex (CPI) of juvenile and subadult quails. These regions are functionally linked to sensory processing, olfaction, and associative learning (Kubota *et al.,* 2020; Zhou *et al.,* 2022). The presence of SOX2-ir cells in these areas suggests that ongoing neurogenic processes may contribute to the maintenance and adaptation of neural circuits involved in sensory perception and learning during early postnatal development.

In the adult brain, a shift in the distribution of SOX2-ir cells was observed, with a low density in the dorsal hippocampus and an absence of SOX2-ir cells in the ventral area. This reduction in SOX2 expression may reflect decreased neurogenic activity and a transition towards more stable neuronal populations in the adult hippocampus. The decline in SOX2-ir cells in the adult hippocampus may signify the earlier recognized completion of developmental neurogenesis (Mercurio *et al.,* 2019) and the establishment of mature neuronal networks involved in cognitive functions. Overall, the distribution of SOX2-ir cells in the hippocampus of juvenile, subadult, and adult quail brains suggests changes in neurogenic activity and progenitor cell proliferation across different developmental stages, shedding light on the regulation of neural plasticity and circuit formation in this brain structure.

Further investigations are warranted to evaluate the molecular mechanisms underlying SOX2 expression and its functional implications in hippocampal development and function in avian species.

The subpallium, which encompasses various nuclei and structures integral to emotion regulation, reward processing, and motor control (Akhmadeev *et al.,* 2019; Akhmadeev *et al.,* 2020), demonstrates distinct patterns of SOX2 immunoreactive (SOX2-ir) cell distribution across different developmental stages in quail brains. In the juvenile and subadult brain, a high density of SOX2-ir cells is observed in several subpallial regions, including the medial and lateral septum (SM and SL), nucleus posterioris amygdalopalli (POA), nucleus accumbens (Ac), fasciculus prosencephali lateralis (FPL), ventral tegmental area (VTA), TnA, and globus pallidus (GP). These regions are integral to the regulation of emotional responses (Pessoa *et al.,* 2019), motivation, and motor behavior (Pessoa *et al.,* 2022).

The presence of a high density of SOX2-ir cells indicates active neurogenic processes and ongoing cellular proliferation within these subpallial structures during early postnatal development, potentially contributing to the maturation and plasticity of the neural circuits involved in these functions. In contrast, the adult brain exhibits a shift in the distribution of SOX2-ir cells within the subpallium. While the lateral septum and nucleus accumbens retain a high density of SOX2-ir cells, other subpallial structures, including the medial septum, show a decrease in SOX2-ir cell density, with low levels observed across all regions. This reduction in SOX2 expression in the adult subpallium may reflect a decline in neurogenic activity and cellular turnover associated with the completion of brain development and the establishment of mature neural circuits (Zocher and Toda, 2023).

However, the persistence of SOX2-ir cells in regions such as the lateral septum and nucleus accumbens suggests ongoing neurogenic processes and potential structural plasticity in specific subpallial areas implicated in reward processing and behavioral regulation. Overall, the differential distribution of SOX2-ir cells in the subpallium across juvenile, subadult, and adult quail brains highlights the complex nature of neurogenesis and cellular proliferation in subcortical structures involved in emotional and motivational behaviors (Pessoa *et al.,* 2019).

The distribution of SOX2 immunoreactive (SOX2-ir) cells within the diencephalon of quail brains exhibits dynamic changes across various developmental stages. This is indicative of variations in neurogenic activity within this brain region (Ngwenya *et al.,* 2018). In the juvenile quail brain, a high density of SOX2-ir cells is predominantly localized within the epithalamic nuclei, particularly in the medial and lateral habenula.

These nuclei are crucial in regulating behaviors such as stress responses, reward processing, and learning (Hikosaka *et al.,* 2020; Li *et al.,* 2021). The presence of a high density of SOX2-ir cells suggests active neurogenic processes and cellular proliferation in these regions during early postnatal development, potentially contributing to the maturation and plasticity of the neural circuits involved in these functions. Additionally, the hypothalamic nuclei display well-expressed SOX2-ir cells scattered throughout the juvenile and subadult brains, with the highest density observed in key regions such as the preoptic nucleus (POM), ventromedial hypothalamus (VMN), tuberal nucleus (TU), median eminence (ME), and anterior medial nucleus of the hypothalamus. These areas are involved in regulating various physiological processes, including temperature regulation, feeding behavior, and hormone secretion (Saper *et al.,* 2002). The observed SOX2-ir cell density suggests ongoing neurogenesis and cellular turnover within these hypothalamic nuclei, possibly contributing to the maintenance and plasticity of the neuroendocrine circuits involved in homeostatic control.

Furthermore, SOX2-ir cells are also observed in the paraventricular nucleus of the hypothalamus (PVN), exhibiting distinctive characteristics such as large cell size (Chen *et al.,* 2022) and cytoplasmic extensions directed towards the anterior commissure (Geerling *et al.,* 2010). These cells displayed the highest density in the subadult brain, indicating active neurogenic processes within this region during early postnatal development. In the thalamus, a moderate density of SOX2-ir cells is observed in various thalamic nuclei, including the ventral lateral geniculate nucleus (GLv), nucleus dorsolateralis anterior thalami (DLAl), nucleus dorsomedialis anterior thalami (DLAm), nucleus rotundus (Rt), and nucleus ovoidalis (OV).

These thalamic nuclei are involved in relaying sensory information to higher brain regions and coordinating sensorimotor integration (Jones, 2007). The presence of SOX2-ir cells in these regions suggests ongoing neurogenic activity and cellular turnover within the thalamus, possibly contributing to the refinement and plasticity of the sensory processing circuits during development. Overall, the distribution of SOX2-ir cells in the diencephalon reflects the dynamic nature of neurogenesis and cellular proliferation in the key regulatory and sensory processing regions of the quail brain. Further research is warranted to elucidate the functional significance of SOX2 expression and its role in the development and plasticity of the diencephalic circuits in avian species.

In the juvenile and subadult quail brain, moderate-density SOX2-immunoreactive (SOX2-ir) cells are observed in various mesencephalic nuclei, including the nucleus semilunaris (SLu), oculomotor nucleus (OMN), Edinger–Westphal nucleus (EW), griseum centrale (Gct), nucleus of the basal optic root (nBor), formatio reticularis medialis mesencephali (FLM), nucleus mesencephalicus lateralis, and pars dorsalis (MLd). These nuclei are involved in the regulation of various motor functions, including eye movement, visual processing, and coordination of motor responses.

The presence of SOX2-ir cells in these regions suggests ongoing neurogenic activity and cellular turnover, potentially contributing to the maintenance and plasticity of motor circuits during development. In contrast, low-density SOX2-ir cells are observed in other mesencephalic nuclei, such as the nucleus isthmi, pars magnocellularis (Imc), nucleus isthmi, pars parvocellularis (Ipc), nucleus intercollicular (Ico), and substantia nigra. The lower density of SOX2-ir cells in these regions may reflect reduced neurogenic activity or cellular proliferation compared with other mesencephalic nuclei.

In the optic tectum, which plays a central role in visual processing and sensorimotor integration in birds, high-density SOX2-ir cells have been observed in the tectal ventricle stratum opticum (SO), stratum griseum centrale (SGC), and stratum album centrale (SAC). These layers are involved in receiving and processing visual information from the retina and integrating it with motor outputs (ref). The presence of SOX2-ir cells in these layers suggests ongoing neurogenesis and cellular turnover, possibly contributing to the development and plasticity of visual-processing circuits during early post-natal development. Overall, the distribution of SOX2-ir cells in the mesencephalon and optic tectum reflects the dynamic nature of neurogenesis and cellular proliferation in regions involved in motor control, sensory processing, and visual integration in the quail brain. Further research is needed to elucidate the functional significance of SOX2 expression and its role in the development and plasticity of mesencephalic and tectal circuits in avian species.

The distribution of SOX2 immunoreactive (SOX2-ir) cells within the cerebellum of quail brains reveals distinct patterns of cellular proliferation and SOX2 expression across various layers and developmental stages. In juvenile and subadult brains, the cerebellar pial surface demonstrates the highest density of SOX2-ir cells, indicating active neurogenic activity and cellular proliferation at the outermost layer of the cerebellum.

This activity likely contributes to the expansion and development of cerebellar structures during early postnatal development. Within the cerebellar layers, including the Purkinje cell layer (PCL) and molecular layer (MCL), a moderate density of SOX2-ir cells is observed in both juvenile and adult brains.

The presence of SOX2-ir cells in these layers suggests ongoing neurogenesis and cellular turnover, which may play a role in the maintenance and plasticity of cerebellar circuits involved in motor coordination and learning (Salih et al., 2022). In contrast, the granule cell layer (GCL) exhibits a lower density of SOX2-ir cells in juvenile and subadult brains, with a moderate density observed in the adult brain. The GCL contains granule cells, the most abundant neuronal population in the cerebellum, which play a crucial role in integrating sensory information and regulating motor output (Welniarz et al., 2021). The observed changes in SOX2 expression in the GCL across different developmental stages may reflect variations in neurogenic activity and cellular proliferation during cerebellar maturation.

Additionally, the nucleus cerebellum internus (CBI) and the internal lining of the recess of the fourth ventricle exhibit moderate densities of SOX2-ir cells, except in the adult brain, where the density is lower. These regions are associated with the regulation of motor functions and coordination, suggesting that ongoing cellular turnover and plasticity in cerebellar structures are involved in motor control (Ngwenya *et al.,* 2018; Welniarz *et al.,* 2021; Salih *et al.,* 2022). Overall, the distribution of SOX2-ir cells in the cerebellum reflects the dynamic nature of neurogenesis, gliogenesis, and cellular proliferation in different layers and regions of the cerebellum across various developmental stages in the quail brain. Further investigation is warranted to elucidate the functional significance of SOX2 expression and its role in cerebellar development and function in avian species.

5.2 Distribution and characterization of PCNA immunoreactive cells in the quail brain

Proliferating Cell Nuclear Antigen (PCNA) is an essential marker for identifying and understanding proliferative activity in the nervous system (González-Magaña and Blanco, 2020; Liu *et al.*, 2020; Arbel *et al.*, 2021). In this study, we employed proliferating cell nuclear antigen (PCNA) to characterize the expression and distribution of cell proliferation within the brains of juvenile, subadult, and adult quails. Our findings indicated that PCNA-immunoreactive (PCNA-ir) cells were expressed in a consistent manner across all brain regions in the various age groups. However, a notable distinction was observed in cell density, which was occasionally absent in the adult group. This observation aligns with age-related studies conducted on chicks and quails (Neves *et al.,* 2007; Nkomozepi *et al.,* 2018b; Wei *et al.,* 2020). The observed high PCNA expression in the olfactory bulb of both juvenile and subadult quail brains, which was reduced to low levels in adults, aligned with findings from Lim and Alvarez-Buylla (2016), who reported that the olfactory bulb remains a major site of neurogenesis throughout life, with a decline in proliferative activity as the brain reaches maturity.

This persistently diminishing neurogenesis is important for olfactory learning and adaptation (Lledo and Valley, 2016), as new neurons are continuously integrated into the existing circuit. Research has shown similar results in canaries (*Serinus canaria*), pigeons, and ring doves (*Streptopelia risoria*), where cells positive for bromodeoxyuridine and 3H-thymidine were identified in the brain (Alvarez-Buylla *et al.,* 1998; Vellema *et al.,* 2010). In the telencephalon, the lateral ventricles (LV) maintain the highest PCNA expression across all developmental stages, reflecting their role as a neural stem cell niche.

This corroborates with the work of Lim and Alvarez-Buylla (2016), who identified the lateral ventricles as a primary source of new neurons, particularly in the adult brain. The lateral wall of the subventricular zone (SVZ) exhibits non-uniformity, characterized by occasional clusters of proliferating cells along the rostrocaudal axis of the lateral ventricle in both species, despite the presence of cell proliferation throughout the lateral ventricle walls. These clusters of proliferating cells are reminiscent of the proliferative hotspots identified in the canary brain (Alvarez-Buylla, 1990; Vellema *et al.,* 2010).

Various avian species demonstrate proliferative hotspots at distinct locations along the rostro-caudal extent of the lateral ventricle (Ling *et al.,* 1997; Vellema *et al.,* 2010; Melleu *et al.,* 2013; Mazengenya *et al.,* 2017; Mazengenya, 2018; Mazengenya *et al.,* 2020). Our observations indicate the presence of cell clusters and aggregations of PCNA-ir cells in the dorsal, medial, and ventral regions of the ventricular wall of the lateral ventricle in juvenile and subadult birds, with a reduced frequency in adult birds. This finding is consistent with observations in adult pigeons, parrots, ostriches, emus, and canaries (Alvarez-Buylla, 1990; Mazengenya *et al.,* 2017; Mazengenya, 2018; Mazengenya *et al.,* 2020; Mazengenya *et al.,* 2022), suggesting the existence of cell clusters in adult birds. Notably, similarities are observed between the juvenile quail cell clusters in our study and those reported by Mazengenya *et al.* (2020) in the common ostrich and emu, where proliferative hotspots were identified at the dorsal and ventral poles of the lateral wall of the lateral ventricle at rostral levels, while the common ostrich exhibited them in the intermediate sections.

These "hot spots" correspond to VZ regions abundant in radial glial cells and are proximal to areas where immature migratory neurons initially emerge (Alvarez-Buylla *et al.,* 1998). In contrast, regions such as the pallium (HA, HI, and HD) and mesopallium (M), including the hippocampal areas (HP), showed a decrease in PCNA expression from juveniles to adults.

This hippocampal decline is consistent with Von Bohlen Und Halbach (2011), who demonstrated that neurogenesis in the hippocampus decreases significantly with age, affecting cognitive flexibility and memory functions. The subpallium regions, including the medial septum (Mst) and lateral septum (Lst), display moderate to low PCNA expressions in juveniles but are absent in adults. This decrease aligns with studies on amphibians by Raucci *et al.* (2006), which suggest that the neurogenic activity of the septal nuclei contributes to the modulation of emotional and cognitive processes during early development but stabilizes in adulthood.

The mesencephalon and rhombencephalon, including areas such as the oculomotor nucleus (OMN) and cerebellum, exhibit high PCNA expression during the juvenile stages, decreasing to moderate or low in adults. This trend is consistent with the role of the cerebellum in motor control and cognitive function, supported by continuous neurogenesis during early development. The reduction in PCNA expression reflects the maturation of the cerebellum and establishment of stable neural circuits. Our findings agree with the broader literature on neurodevelopment and neurogenesis. The decrease in PCNA expression from the juvenile to adult stages highlights a shift from high proliferative activity to the stabilization of neural networks.

This transition is a critical aspect of brain maturation, emphasizing the importance of refinement in achieving functional neural systems (Nkomezepi *et al.,* 2018; Mazengenya *et al.,* 2018, 2020). The differential expression of PCNA across brain regions and developmental stages has significant implications in understanding the mechanisms of neural function and potential regenerative therapies. This observation suggests that manipulating neurogenic niches could offer therapeutic benefits.

A detailed analysis of PCNA expression patterns provides a comprehensive understanding of neurogenic potential and maturation of the nervous system. These findings, supported by the existing literature, underscore the importance of regulated cellular proliferation in brain development and highlight the potential for therapeutic interventions targeting neurogenic processes in neurological disorders.

The high expression of PCNA in the OMN during the juvenile and subadult stages indicated proliferative activity, suggesting that active cell proliferation is critical during these early stages for the maturation of motor control systems. The OMN, responsible for controlling eye movements and coordination of extraocular muscles, undergoes significant developmental refinement to ensure the precise motor outputs required for complex visual-motor tasks (Schor, 2011). The reduction in PCNA expression from the juvenile to adult stages indicates a decrease in proliferative activity as the region matures. This decline aligns with the literature, such as the findings of Marín-Padilla, (1998), which described that during development, motor nuclei involved in fundamental sensory-motor pathways undergo significant neural differentiation and synaptic pruning, reducing the need for continuous neurogenesis in adulthood.

As neural circuits in the OMN stabilize, the role of PCNA as a marker of cell proliferation diminishes, indicating a shift from growth to functional maintenance. Similarly, high PCNA expression in the Red Nucleus (RMC) during the juvenile and subadult stages highlights its critical role in motor development. The Red Nucleus is involved in motor coordination, particularly in facilitating skilled limb movement. During early development, neurogenesis in this region is essential for establishing the descending rubrospinal tract for fine motor control and voluntary movement. This finding is consistent with the existing knowledge (Marani, 2024), which showed that the Red Nucleus continues to develop through early life stages as new synapses form and neural circuits refine.

The decline in PCNA expression to moderate levels in adulthood suggests a reduction in proliferative activity once these circuits mature. However, the fact that moderate PCNA expression persists into adulthood indicates that some degree of plasticity or repair capacity may remain. This observation corresponds with studies by Yamamoto *et al.* (2001), who found that adult neurogenesis in motor-related areas can contribute to the repair and reorganization of motor circuits following injury or neurodegenerative conditions.

The high-to-moderate decline in PCNA expressions in both the OMN and RMC reflects the patterns observed in other motor control regions. According to Altman and Bayer (2002), motor nuclei exhibit early life neurogenesis that gradually tapers off as the brain's motor systems fully mature. This is particularly important in regions such as the Red Nucleus and Oculomotor Nucleus, where precise motor functions such as eye and limb movements rely on well-established and stable neural networks.

While neurogenesis or gliogenesis increases in these regions diminish, studies by (Yamamoto *et al.* (2001) indicated that certain motor-related areas retained some capacity for cell proliferation in response to external stimuli or damage, which could explain the moderate PCNA expression observed in adults (inement in achieving functional neural systems (Nkomezepi *et al.,* 2018; Mazengenya *et al.,* 2020).

During the early developmental stages, these regions require high levels of cellular proliferation to establish neural circuits that are essential for motor function. As circuits mature, proliferative activity is reduced, and both neurogenesis and gliogenesis become more focused on maintaining function rather than building new networks. However, the persistence of moderate PCNA expression in adults suggests that these areas retain some capacity for neuroplasticity, particularly in response to injury or other neural disruptions.

High PCNA expression in the cerebellar intermediate layer (Cbi) in both juvenile and subadult stages indicates robust cell proliferation during early life. This high expression level corresponds to ongoing neurogenesis and gliogenesis in this region, supporting the formation and refinement of cerebellar circuits that are crucial for motor coordination and sensory processing.

The reduction in PCNA expression in the Cbi to low levels in adults suggests that the primary phase of neural development in this layer is largely completed by adulthood. This decline is consistent with the findings of Rueda-Alaña and García-Moreno (2022), who reported that cerebellar neurogenesis peaks during early development and then declines as motor control systems stabilize. As the neural circuitry in the cerebellum becomes more established and specialized, the demand for proliferative activity decreases, leading to reduced PCNA expression.

The expression of PCNA in the Purkinje cell layer (Pcl) of juveniles is indicative of active cell proliferation and synaptogenesis during early developmental stages, but it decreases to moderate density in subadults and further reduces in adults.

The gradual reduction in PCNA expression in the Pcl with age aligns with the existing literature (van der Heijden *et al.*, 2021), which describes the early life proliferation of Purkinje cells, followed by synaptic pruning and maturation of these cells as cerebellar circuits are refined. By adulthood, Purkinje cells establish extensive dendritic arborizations and functional synaptic connections, reducing the need for continuous cell proliferation. The low to negligible levels in the molecular cell layer (McL) could be because the layers are mainly composed of synapses and dendritic processes from Purkinje cells with relatively fewer neurons. Thus, its presence indicates gliogenesis only. As described by Sawada and Kamiya (2023), the cerebellar molecular layer in rodents undergoes substantial synaptic organization during early development but requires minimal neurogenesis. The layer integrates synaptic inputs rather than producing new cells.

The granule Cell Layer (Gcl): The low levels in the granular layer could be because early proliferation is critical for establishing cerebellar circuitry. However, neurogenesis is primarily confined to early developmental periods, tapering off as the cerebellum matures (Rueda-Alaña and García-Moreno, 2022). This pattern of declining PCNA expression is supported by a study by Espinosa and Luo (2008) who found that granule cell neurogenesis occurs primarily during early postnatal development and decreases significantly in adulthood. This is also the case for the pigeons. emu, ostrich, and quail (Nkomezepi *et al.,* 2018; Mazengenya *et al.,* 2018, 2020., Harold *et al.,* 2023).

By adulthood, most granule cells have already been generated, and cerebellar circuits become stable, reducing the need for further neurogenesis.

The overall trend of declining PCNA expression across cerebellar layers as the organism matures is consistent with broader findings on cerebellar development. van der Heijden *et al.* (2021), Rueda-Alaña and García-Moreno (2022), Sawada and Kamiya (2023) highlighted that neurogenesis in the cerebellum peaks during early development and is crucial for motor function maturation. By adulthood, the role of the cerebellum shifts from growth to maintaining its complex circuitry, with limited neurogenesis occurring primarily in response to specific stimuli. Moreover, studies have emphasized the critical role of Purkinje cells and their early development in motor coordination (van der Heijden *et al.*, 2021). The PCNA expression patterns in this study support the notion that the Purkinje cell layer undergoes significant refinement during the early stages, with reduced neurogenesis as functional circuits stabilize (Mazengenya *et al.,* 2018, 2020; Nkomezepi *et al.,* 2018)., Harold *et al.,* 2023). The results underscore the fluctuating nature of PCNA expression in various brain regions throughout development. The variations in PCNA levels from juvenile stages to adulthood indicate a transition in neurogenic capacity and cell proliferation as the nervous system evolves.

Gaining an understanding of this expression patterns could offer valuable insights into the mechanisms of brain development and may have implications for future studies on neuroplasticity and age-related neurological disorders.

5.3 Distribution and characterization of GFAP immunoreactive cells in the quail brain.

This study demonstrated the distribution of astrocytes in the brains of juvenile, sub-adult, and adult Japanese quails. The presence of astrocytes labelled with the GFAP antibody was observed in the olfactory bulb, telencephalon, pial surface, diencephalon, mesencephalon, rhombencephalon, third ventricle, and fourth ventricle across the various age groups of the studied quails. This observation is supported by findings from other birds such as songbirds (Zhou *et al*. 2000), zebrafish (Santos-Ledo *et al*. 2023), and other vertebrates (Kettenmann and Verkhratsky 2022). Gliogenesis persists throughout adulthood in the CNS. For example, new neurons are continuously generated in all brain regions of birds, and the stem elements that produce neurons are astroglia (Emsley *et al*. 2005; Grandel and Brand, 2013; Paredes *et al*. 2016, Kempermann 2022). Building upon these observations, this study also revealed distinct classes of astrocytes with specialized functions. This investigation also revealed the presence of a distinct class of astrocytes comprising intensely stained unipolar cells lining the inner surface of the pia mater, perivascular astrocytes found in large blood vessels, and another type in the hippocampus.

These astrocytes represent a specialized type of radial, predominantly observed in the juvenile brain. They are responsible for maintaining the structure and function of specific neurons found in these regions. Furthermore, they play a role in maintaining neurotransmitter homeostasis, preventing the efflux of certain neurotransmitters, such as glutamate, to other regions, and inhibiting peripheral glutamate from reaching these brain regions.

Comparable findings were also reported by Verkhratsky and Nedergaard (2018) and Kempermann (2022), who independently noted that radial glia and astrocytes contribute to neurogenesis and the maintenance of homeostasis in various brain regions.

In addition to these specialized astrocytes, the study also examined the distribution of GFAP-immunoreactive cells across different age groups and brain regions. Similarly, there was a higher distribution of GFAP-immunoreactive cells on the pial surface across all age groups in juveniles, subadults, and adults, which is consistent with the findings of Kálmán *et al*. (1993) in domestic chickens (Gallus domesticus), where GFAP-immunoreactive cells were present in substantial quantities on the pial surface. The high density of these cells in the pial surface has been attributed to the presence of blood vessels around the pial surface; consequently, astrocytic bodies are present to provide end feet with arterial blood vessels, thereby forming a delicate blood-brain barrier (BBB). Additionally, astrocytes in the area serve as a point of proliferation for new astrocytes (gliogenesis), particularly in juveniles and subadults, which are in the form of radial glia, and the pial surface serves as a site of adult neurogenesis, particularly in adults. These assertions are supported by the works of Verkhratsky and Nedergaard (2018), and a subsequent study by Verkhratsky *et al*. (2021) where it was also posited that astrocytic end feet are formed with the blood vessels around the pial surface of numerous vertebrates including mammals (mice). Other research has reported similar findings in songbirds (López-Murillo *et al*., 2024), domestic fowls (Kálmán *et al*., 1993), zebrafish (Balakrishnan 2007, Johnson *et al*., 2016, Lee *et al*., 2017, Kálmán *et al*., 2021). While the pial surface showed a high density of GFAP-immunoreactive cells, other types of astrocytes were observed in different brain regions

The second type of astrocyte observed in this study was represented by multipolar astrocytes of variable sizes, with an irregular cell body found in the ventricular wall and large fiber tracts. Similar elements represent the last type, exhibiting an immunonegative cell body that can be identified only by converging processes scattered within white matter. These three types of cells and several isolated processes demonstrate differential distribution within the quail brain, both in the gray and white matter. The study not only identified different types of astrocytes but also examined their distribution in specific brain areas, particularly in the ventricular regions.

In the present study, the lateral, third, and fourth ventricles exhibited a high density of immunoreactive GFAP cells. These cells were more prominent in the subventricular zone (SVZ) of all ventricular regions. These regions are known to proliferate glial (astroglia) cells as well as new neuronal cells across all age groups. These cells predominantly have similar structures, possessing star-like and radial shapes. Those with radial shapes are known as radial glia, which are primarily predominant in juveniles, whereas the more mature star-like shaped astrocytes are more prevalent in adults. However, radial glia was found in both adults and juveniles, which is consistent with the earlier work of Kálmán *et al*. (1993), where it was demonstrated that in birds, unlike mammals, radial glia persist into adulthood, forming a substantial part of glial architecture. This phenomenon has also been observed in other regions, such as the telencephalon, as confirmed by a more recent study by the same author (Kálmán and Sebők 2023). GFAP immunoreactive cell density was observed to have a moderate to low density in the pons and medulla, and there was a decline in GFAP-positive elements in the 12-week adult quail, which is consistent with the work of Verkhratsky *et al*. (2021).

They posited that the capacity of astrocytes to support axonal growth diminishes with age; embryonic astrocytes strongly support axonal growth, whereas mature astrocytes inhibit axonal growth. Consequently, the astroglial scar that form following damage to the adult CNS presents a significant barrier to axonal regeneration. Furthermore, it has been observed that, after neurons reach their final sites during maturation, they extend axons, which in some instances grow for considerable distances and must cross the brain midline, decussating to reach their synaptic targets (Verkhratsky and Nedergaard 2018, Ray *et al*. 2020). Channels formed by astrocytes provide mechanical and guidance substrates for axonal growth (Damo and Tedeschi 2020, Damo and Simonetti 2022). In the corpus callosum, astrocytes form a bridge (glial sling) that connects the left and right sides of the developing telencephalon (Dysgeneses 2019; Raybaud 2019).

5.4 Distribution and characterization of S100A1 immunoreactive cells in the quail brain

The text examines the distribution of the protein S100A1 among various vertebrate groups. It seeks to compare how this protein is present and distributed across avian, mammals, reptiles and amphibians. The distribution pattern of the S100A1 protein in the brain of the Japanese quail reveals varying levels of expression across different regions, ranging from high to moderate to low. S100A1, a calcium-binding protein found in astroglial cells, plays a role in calcium signaling, neuroprotection, and maintaining cellular balance. These expression patterns underscore the functional importance of this protein in distinct brain areas. In the olfactory bulb, S100A1 expression was either absent or minimal. This low expression level suggests that astrocytes expressing S100A1 are probably not involved in olfactory processing in quails.

In mammals, S100 proteins have been linked to olfactory neurogenesis, where astrocytes activated by olfactory stimuli are associated with neural plasticity and synaptic remodeling (Bailey & Shipley, 1993; Ladher *et al.,* 2011; Cuentas-Condori & Miller, 2020; Silvas-Baltazar *et al.,* 2023; Bovetti *et al.,* 2024). This lack of expression in quails contrasts with findings in amphibians and reptiles, where glial markers are prominent in the olfactory bulb due to their role in neurogenesis (Lazzari & Franceschini, 2001; González-Granero *et al.,* 2011; Powers, 2016; Cooper, 2016; Liberia *et al.,* 2024). Differences among vertebrates may include variations in regeneration processes and the involvement of astrocytes in sensory information processing.

The pallium exhibited regional variations, with moderate-to-high levels of S100A1 expression in HP and HA, while other pallial regions showed little to no expression. In the subpallium, areas like the medial striatum (Mst) and lateral striatum (Lst) displayed high expression levels. The elevated expression in the pallium (HP, HA) is linked to its crucial role in spatial memory and navigation. In mammals, hippocampal astrocytes play a key role in regulating synaptic plasticity and memory consolidation (Miyamoto *et al.,* 2017; Pinto-Duarte *et al.,* 2019; Cho *et al.,* 2022; Yang *et al.,* 2022).

The involvement of astrocytes in both avian and mammalian hippocampi underscore the conserved function of S100A1 in managing synaptic activity. In the subpallium, the high expression in the striatum suggests astrocytic participation in motor control and reinforcement learning. S100A1 is crucial for dopamine-glutamate interaction in the basal ganglia circuits in mammals (Donato *et al.,* 2013).

In reptiles and amphibians, glial support in the striatum is vital for sensorimotor integration (Broglio *et al.,* 2003), indicating a conserved role across vertebrates. In the diencephalon, which includes the Hypothalamus and Thalamus, S100A1 expression was observed at moderate to high levels in the hypothalamic regions, with the preoptic area (POM) and paraventricular nucleus (PVN) showing relatively higher expression. The thalamic areas (Rt and OV) exhibited moderate expression levels. This elevated expression is likely due to the hypothalamus's role in managing neuroendocrine signaling, homeostasis, and reproductive behaviors. It is believed that hypothalamic astrocytes influence hormone activity in mammals (Brancaccio *et al.,* 2014, Brancaccio *et al.,* 2019, Micevych & Mohr, 2021; Velloso *et al.,* 2022). A similar function for astrocytes has been suggested in amphibians concerning seasonal reproductive changes (Dey & Karar, 2023; Rastogi *et al.,* 2002).

The similarity in distribution in quails implies conserved astrocytic functions in neuroendocrine regulation. Moderate expression in the thalamus (Rt, OV) suggests involvement in sensory relay functions. Due to the need for processing and evaluating sensory information, S100A1 expressed in thalamic astrocytes regulates attention mechanisms (Zimmer *et al.,* 1998; Müller *et al.,* 2018; Jebessa *et al.,* 2022, 2023) in mammalian studies.

Similar findings in birds highlight the evolutionary significance of astrocytic modulation in thalamocortical circuits. S100A1 expression was moderate in the optic tectum and highest in the midbrain structures (OMN and RMC). Moderate expression in the optic tectum, the primary site of visual processing in birds, suggests that astrocytes in this region are involved in visual information processing.

Astrocytes in the mammalian superior colliculus modulate the visual-motor response (Schummers *et al.,* 2008). In amphibians and reptiles, tectal glial cells are essential for visual plasticity (Gaze *et al.,* 1990), indicating a conserved function. The cerebellum exhibited strong S100A1 expression in the Purkinje and granular layers (Cbi, MI, PcI) and moderate expression in the Rpgc, region of the pons.

In the cerebellum, high expression in Purkinje cell layers corresponds to astrocytic-independent roles in motor coordination and learning. The role of S100A1 in Bergmann glia is to maintain calcium homeostasis, crucial for synaptic plasticity in mammals (Bayghen *et al.,* 2007; Andriezen *et al.,* 2010; Xu suggested 2013; Shuvaev *et al.,* 2021; Edamakanti *et al.,* 2022).

Similar studies in reptiles and amphibians suggest that astrocytes may play similar roles in these species, further emphasizing the importance of S100A1 in motor learning across taxa (Duarte-Costa *et al.,* 2014). Moderate expression implies astrocytic involvement in motor and autonomic control (Goursaud *et al.,* 2013). S100A1 expressing brainstem astrocytes play a role in respiratory and cardiovascular function (Li *et al.,* 2008, Jebessa *et al.,* 2018, Sivasubramanian *et al.,* 2020) as comparative studies of mammals have shown.

The medulla showed moderate to high levels of expression in various nuclei, particularly those involved in autonomic functions. The presence of astrocytes in the medulla suggests their possible role in influencing autonomic activities, such as respiratory and cardiovascular functions. In mammals, medullary astrocytes are involved in synaptic organization within brainstem reflex circuits (Las Heras *et al.*, 2022) (Gourine *et al.,* 2010). A similar pattern observed in birds suggests a conserved role for astrocytes in autonomic regulation across vertebrates.

Additionally, different expressions of S100A1 during various growth stages can impact avian behaviors and cognition. The elevated expression of S100A1 in juvenile and sub-adult quails, which plays a role in calcium signaling and has neuroprotective effects, might be linked to enhanced opportunities for experiential learning and adaptation to the environment. This is particularly important for survival, as young quails must quickly learn to avoid predators and find food independently.

The distribution pattern of S100A1 can also be associated with its proposed functions in learning and memory, as well as the evolutionary adaptations of birds to avoid predators. High-density S100A1-ir expression in regions associated with sensory processing and motor control indicates that these functions were evolutionarily favored in birds, potentially contributing to their ecological success. For a species that relies heavily on visual cues for navigation and foraging, the ability to rapidly process visual information and respond to environmental stimuli is crucial.

To conclude, the presence and characteristics of the S100A1 protein in the brains of juvenile, subadult, and adult male Japanese quails indicate its significant role in avian neurobiology. The high concentrations found in the subpallium, diencephalon, mesencephalon, and optic tectum during the juvenile and subadult phases suggest its importance in sensory processing, motor coordination, and possibly cognitive functions. The age-related decline in S100A1 highlights its reduction in relation to neural maturation, behavioral recovery, and adaptability. Collectively, these observations offer a detailed understanding of the neurobiological basis of avian development and behavior, with potential implications for future research. This study also underscores the necessity for further investigations into the molecular mechanisms that control S100A1 expression.

Additionally, exploring the signaling pathways that regulate S100A1 levels during development could provide deeper insights into its role in neurodevelopmental processes.

5.5 Distribution and characterization of OLIG2 immunoreactive cells in the quail brain

The presence of oligodendrocyte progenitor cells has been described in the brains of animals, such as mice (Abraham *et al*., 2023) and zebrafish (Masson and Nait-Oumesmar 2023). Similarly, studies have described their activities in the retina of some songbirds (Seo *et al*., 2001), goldfish (Tibi *et al*., 2023), and zebrafish (Santos-Ledo *et al*., 2023). This widespread presence suggests that they are important for electrical impulse conduction in the nervous system. The results of this study revealed different regional and age-related changes in Olig2 expression in the Japanese quail brain.

The highest density observed in the juvenile and subadult stages and high Olig2 immunoreactivity shown by the telencephalon suggested active oligodendrogenesis during early development (Goldman & Kuypers, 2015). Especially in the hyperpallium and mesopallium, which are linked to sensory processing and cognition, the pallium showed notably high Olig2 expression (Jarvis *et al*., 2013). Consistent with continuous oligodendrogenesis into adulthood, the subventricular zone (SVZ), a known neurogenic and gliogenic niche, maintains Olig2-positive cells across all stages (Alvarez Buylla *et al*., 2018). Olig2-ir cells were observed in the habenula, thalamus, and hypothalamus of the diencephalon with a developmental decrease in expression.

Especially the POM and PVN, the hypothalamic areas showed a peak in Olig2 expression in the subadult stage, implying a link between gliogenesis and hormonal control during maturation (Liao, *et al*., 2025). In the mesencephalon, particularly the optic tectum kept Olig2-ir cells in the SO and SGF layers, in line with results in other avian species where the tectum is crucial for visual processing and sensorimotor integration (P.J Straight *et al*., 2023, Khama, Wael *et al*., 2024, Liao, *et al*., 2025)

The study's findings showed that the optic tectum, optic tract, anterior and posterior commissure, and tectal ventricle, which the cells are arranged in rows, all had high densities of positive Olig2 immunoreactivity (OLIG2-ir). This finding is consistent with the work of Diez (2021) in songbirds, and other studies have suggested that Olig2 is present throughout the vertebrate oligodendroglial lineage (Zhou *et al*. 2000, Zhou and Anderson 2002, Liu *et al*. 2021, Zhang *et al*. 2022).

Olig2 is essential for the development of neural progenitor cells (NPC) into oligodendrocyte progenitor cells (OPC) (Xu *et al*. 2022, Zhang *et al*. 2022, Pieczonka *et al*. 2023) and in vertebrates like mice, remyelination has been linked to Olig2 gain of function in OPC (Xia and Fancy 2021, Wang *et al*. 2022, Ihunwo 2023).

Although it has been reported that oligodendrocytes are absent in some vertebrate retinas, studies carried out by Seo *et al*. (2001) on rabbits and a follow-up study by Diez (2021) on avian retina reported that there exists a large quantity of OPC’s in the retina of these vertebrates, after staining positive for olig2 immunoreactivity. Since the intraretinal axons of the ganglion cells in birds are myelinated and the optic fiber axons are myelinated, the activity of oligodendrocytes in the retina is further transmitted to the optic tract of songbirds and other avian species.

This allows for fast transmission along the optic nerve fibers (Suminaite *et al*. 2019; Diez 2021; Kuloglu 2022; and Quintela-López *et al*. 2022). Especially in white matter areas such as the cerebellar peduncles, which support motor coordination, the rhombencephalon displays strong Olig2 expression in the cerebellum (Sultan & Glickstein, 2022). Important for motor refinement, the higher density of Olig2-ir cells in the subadult cerebellum points to continuous myelination and neural circuit maturation (Wang *et al*., 2020). The theory that oligodendrogenesis peaks in early development and decreases with age is supported by the decrease in Olig2 expression in the adult brain.

Additional findings from this study demonstrated that, whereas myelinated fiber density increased with developmental stage, it decreased in the adult brain. The areas of the quail brain with the highest myelination levels correlated with the density of OLIG2-ir oligodendrocytes. The fact that juvenile avian oligodendrocytes are typically more abundant than adult oligodendrocytes lend credence to this, possibly because of their active participation in myelination and proliferation throughout the bird's developmental phases (Bhattarai *et al*. 2022, Morizet *et al*. 2024). As juvenile birds mature, the number of oligodendrocytes tends to stabilize, leading to fewer in the adult stage, as many have already completed their growth and myelination processes (Xin and Chan, 2020). This increase in the density of myelinated fibers suggests that oligodendrocyte activities are present and are largely responsible for myelination in the brain regions of quails (Bergles and Richardson 2016, Hide *et al*. 2019, Fowl 2023), the rapid myelination observed in the juvenile brain is crucial for the maturation of neural circuits during peak developmental phases (Monje 2018, Sakai and Sugiyama 2018, Moura *et al*. 2022).

Kuhn *et al*. (2019) and Moura *et al*. (2022) reported that juvenile oligodendrocytes produce trophic factors that support the survival and growth of neighboring neurons. This finding supports its function in promoting neuronal plasticity, which is comparable to that of humans and other vertebrates, as well as its contribution to total brain development (La Rosa *et al*., 2020; Snell-Rood and Snell-Rood 2020; Bonfanti and Charvet 2021).

Understanding the distribution and developmental changes in these cells in the Japanese quail brain provides valuable insights into avian neurobiology. The quail's relatively short lifespan, easily accessible embryo, and physiological similarities to humans make it an excellent model for studying developmental biology, aging, and diseases (Huss *et al*., 2008). Ultimately, the spatiotemporal distribution of Olig2-positive oligodendrocytes in the Japanese quail brain was highlighted in this study, exposing developmental changes to important brain areas. The juvenile and subadult stages showed the highest Olig2 expression; generally, this suggests an early peak in oligodendrogenesis, as seen in adulthood. With notable expressions in neurogenic and myelination-related structures, the telencephalon, diencephalon, mesencephalon, and rhombencephalon show region-specific patterns.

These findings not only contribute to our knowledge of avian neurobiology but also have potential implications for understanding similar processes in other vertebrates, including humans. These results help clarify gliogenesis in avian species and advance our knowledge of relative neural development. Future research should investigate the functional consequences of these cellular modifications, particularly in relation to cognition, motor control, and neuroplasticity.

5.6 Distribution and characterization of IBA-1 immunoreactive cells in the quail brain

The presence of brain macrophages and/or early microglia in the developing brain has been described in all vertebrates including zebrafish (Casano *et al.,* 2016), amphibians (Cuadros *et al.,* 2022), birds (Duncan *et al.,* 2013), rats (Bilimoria and Stevens 2015), mice (Casano and Peri 2015, De *et al.,* 2018) and humans (Tay *et al.,* 2017, Hammond *et al.*, 2018). This widespread presence suggests their importance for normal nervous system development and early sculpting (Cuadros Ojeda *et al*., 2022, Kettenmann and Verkhratsky 2022).

This study revealed three immunoreactive cell types: ameboid microglia (circular, no processes), transitional microglia (elongated bodies, thick/long processes), and ramified microglia (normal bodies, long thin processes). These findings align with several authors (Arnoux *et al.,* 2013; Arnoux and Audinat 2015; Tay *et al.,* 2017, Smolders *et al.,* 2019).

Microglia have been identified in various invertebrate and vertebrate species (Garaschuk and Verkhratsky 2019; Sharma *et al.,* 2021, Sardar *et al.,* 2022). However, ontogeny and maintenance investigations have mainly been in rodents (Sharma *et al.,* 2021), with few studies in birds, especially Japanese quail (Cuadros *et al.,* 1994), focusing only on optic tectum microglia. The absence of IBA-1 immunoreactivity in the olfactory bulb across all age groups suggests a minimal microglial role during examined developmental stages. However, this doesn't necessarily indicate complete lack of involvement. Microglia may adopt a specialized phenotype or express alternative markers undetected by IBA-1 (Guselnikova *et al.,* 2024).

Potential microglial roles in the olfactory bulb include synaptic pruning, neurogenesis, immune surveillance, and neurotrophic support (Colonna and Butovsky 2017). Future studies using advanced techniques can provide further insight into microglia's role in the olfactory bulb. High IBA-1 expression in juvenile and subadult lateral ventricular zones (LV) may reflect ongoing neurogenesis, similar to microglia in proliferative brain regions. This aligns with studies suggesting microglia's active role in neurodevelopmental processes (Sato, 2015; Colonna and Butovsky, 2017). The reduction in IBA-1 expression in adults may indicate a shift from developmental to surveillance function, supporting age-related decreases in neuroplasticity (von Bernhardi and Eugenín, 2024).

Moderate expression in hippocampal juveniles aligns with studies highlighting high microglial density during early postnatal period to support hippocampal development, learning, and memory formation (Paolicelli and Ferretti, 2017). The reduction in subadults and absence in adults aligns with evidence showing that as hippocampal functions establish, microglia decrease in density, transitioning from a formative role to maintaining homeostasis (Russo and McGavern, 2015).

High IBA-1 expression in the hippocampus during early development suggests a critical role for microglia in shaping hippocampal structure and function. Microglial activation in the pallial hippocampal region during subadult stage may suggest transient activity associated with maturation, potentially linked to refinement of neuroplasticity or synaptic stability (Blagburn-Blanco *et al.,* 2022). The absence of IBA-1 in juveniles and adults suggests reduced microglial involvement, supporting studies indicating that APH may not rely heavily on microglial activity under non-pathological conditions (Muzio *et al.,* 2016).

Microglial activation in the anterior pallial hippocampal region during subadult stage may indicate a specific role in the maturation of this region. Low microglial density in the dorsolateral and piriform cortices of subadult brains might indicate a basal surveillance function where microglia remain dormant until activated by injury or pathology. Studies in avian species have suggested minimal microglial involvement in normal development of these regions (Delage and Cornil, 2020), aligning with their potential sensory-processing roles and reduced neurogenesis. Low IBA-1 expression in the dorsolateral and piriform cortices suggest microglia may play a less prominent role in the development and function of these regions than in other brain areas.

Low-to-moderate IBA-1 immunoreactivity in the hyperpallium intercalatum (HI), dorsal hyperpallium (HD), mesopallium (M), nidopallium (N), and entopallium (E) regions suggests a more limited role of microglia than in other pallial areas. The age-related decline in microglial density in these regions aligns with neural circuit maturation and synaptic connection stabilization. As the brain matures, the need for active microglial involvement in shaping neural networks decreases, and microglia transition to a more surveillant role. This is consistent with findings from rodent studies showing decreased microglial density in the cortex and hippocampus during postnatal development (Paolicelli and Ferretti 2017). However, the specific roles of microglia in these pallial regions may vary depending on developmental stage and sub-region. Understanding microglial roles in these regions may provide insight into the pathophysiology of neurodevelopmental and neurodegenerative disorders. High IBA-1 expression in the lateral septum during early developmental stages suggests a crucial role for microglia in shaping neural circuitry.

Microglia may be involved in establishing neural connections underlying social behaviors, stress responses, and emotional regulation. This is supported by studies showing microglia's role in synaptic pruning and neural circuit development in other brain regions (Paolicelli and Ferretti, 2017).

Like the lateral septum, the medial septum exhibited high levels of IBA-1 expression during early development. Microglia likely play a role in synaptic pruning, neurogenesis, and establishment of neural connections for memory and spatial navigation. This aligns with studies showing microglia's involvement in hippocampal development and function, a region closely connected to the medial septum (Lenz and Nelson, 2018). The medial septum, critical for memory and spatial navigation, shows a developmental pattern in IBA-1 expression. During early development, high levels of IBA-1-positive microglia likely support neurogenesis and synaptic maturation. As the brain matures, these processes decline, reducing microglial activity and IBA-1 expression. This aligns with microglia transitioning from active developmental roles to homeostatic functions in adulthood.

In the Hypothalamus, high levels of IBA-1 expression have been observed in the preoptic area of juvenile quail brain, suggesting active microglial involvement in developmental processes. As the brain matures, microglial density decreases, indicating a transition to homeostatic function.

The paraventricular nucleus (PVN), involved in stress response and autonomic regulation, displays high IBA-1 expression in juveniles, suggesting active microglial involvement in establishing neural networks for stress and metabolic control. Microglial density decreases with brain maturation, indicating a reduction in developmental roles.

The ventromedial nucleus (VMN) regulates energy homeostasis and defensive behavior. High IBA-1 expression in juveniles suggests microglia's role in synaptic organization and development of circuits controlling feeding and defense responses. Microglial density decreases as the brain matures. The reticular nucleus (Rt) showed higher IBA-1 expressions in subadults than juveniles, suggesting a potential microglial role in specific developmental processes, such as refinement of thalamocortical connections during adolescence.

This aligns with research highlighting microglia's dynamic role in shaping neural circuits during critical developmental periods. The oval nucleus (OV) exhibited low IBA-1 expression in juveniles, with no expression in subadults or adults, suggesting minimal microglial involvement, especially in later developmental stages. This aligns with reduced microglial involvement in mature brain regions. The Dorsolateral Anterior Thalamic Nucleus (DLA) showed moderate IBA-1 expression in juvenile and subadult stages, slightly decreasing in adults. This suggests microglia's active role in developing cognitive and sensory processing circuits. High IBA-1 expression in juveniles and subadults aligns with known microglial roles (Lenz and Nelson, 2018; Xu et al., 2021).

The medial septum exhibited high IBA-1 expression during early development. Microglia likely play a role in synaptic pruning, neurogenesis, and establishment of neural connections underlying memory and spatial navigation. The Dorsomedial Anterior Thalamic Nucleus (DMA) showed high IBA-1 expression in juvenile and subadult stages, decreasing in adults. This aligns with microglia's role in thalamic sensory processing area maturation.

High microglial activity in early development suggests a crucial role in shaping neural circuits for sensory integration and motor control, consistent with studies on microglia in neurodevelopmental disorders (Salter and Stevens, 2017). The Geniculatus Lateralis Pars Ventralis (Glv), involved in visual information processing, displayed decreasing IBA-1 expression over time. This likely reflects thalamic visual processing circuit maturation, where microglial activity is essential for early synaptic pruning and circuit refinement. This observation aligns with studies highlighting microglia's role in sensory system development during critical plasticity periods (Sato, 2015; Lenz and Nelson, 2018).

The mesencephalon, crucial for motor coordination, visual processing, and sensory integration, exhibits varying microglial expression patterns across age groups.
The Oculomotor Nucleus (OMN), controlling eye movements, showed high IBA-1 expression in juvenile and subadult stages, decreasing in adulthood. This suggests microglia's dynamic role in ocular motor coordination development, likely involving synaptic pruning and circuit refinement, consistent with previous studies (Hoshiko et al., 2012).

The optic tectum, critical for visual processing, exhibits high IBA-1 expression in juveniles, decreasing in subadults and adults. This suggests microglia's key role in early visual circuit maturation, including synaptic pruning and refinement (Clark et al. 2015). As the brain matures, microglial activity decreases, reflecting a transition to a homeostatic role. Stratum Griseum et Fibrosum Superficiale (SGFS) and Stratum Griseum Fibrosum (SGF), involved in sensory processing and integration, also showed high IBA-1 expression in juveniles, gradually decreasing in subadults and adults.

This suggests microglia's role in synaptic remodeling during early development (Ginhoux *et al.,* 2013). Age-related reductions in microglial density likely correspond to synaptic connection stabilization. The Stratum Album Centrale (SAC) exhibited low levels of IBA-1 expression across all age groups, suggesting a less active role for microglia in this region than in other areas of the mesencephalon. This may reflect a less dynamic role in synaptic remodeling and circuit formation, consistent with the more stable nature of SAC function.

Cerebellar internal layer (Cbi): The absence of microglial expression in the Cbi across all age groups suggests a lower requirement for microglia-mediated synaptic remodeling and plasticity. This aligns with the cerebellum's primary function in motor coordination, which may be less dependent on microglial involvement in synaptic plasticity (Ginhoux *et al.,* 2013).

Molecular cell layer (Ml): High IBA-1 expression in juveniles and subsequent decrease in subadults and adults suggest a role for microglia in synaptic refinement during early development. This aligns with microglia's known role in shaping neural circuits during critical developmental periods (Carrasco et al. 2011, Marín-Teva *et al.,* 2011).

Purkinje cell layer (Pcl): Mild IBA-1 expression in juveniles and its absence later indicates a potential role for microglia in maturing synaptic connections between Purkinje cells and target neurons. Granule Cell Layer (Gcl): The absence of IBA-1 expression suggests microglia may play a less significant role in this layer's development and maintenance.

Nucleus VIIIc (NVIIIc): High IBA-1 expression in juveniles and subsequent decrease in adults suggest a role for microglia in synaptic pruning and neuroplasticity during vestibular function development. This aligns with microglia's role in shaping neural circuits during critical developmental periods (Hanisch and Kettenmann 2007). The minimal IBA-1 expression observed across all age groups suggests a less active role of microglia in this region, likely reflecting a more passive role in immune surveillance. This is consistent with microglia in the medulla having a more homeostatic role in maintaining tissue health and responding to potential insults.

5.7 Distribution and characterization of DCX-ir in the quail brain

In the telencephalon, the observed DCX pattern in the juvenile and subadult species indicates active neurogenesis and neuronal migration during these developmental stages, confirming DCX's role in marking new and migrating neurons (Saaltink et al., 2012). The regional variation, which includes higher densities in the Hyperpallium apicale (HA), lower densities in the core, and a front-to-back gradient, suggests differential neurogenic activity and developmental timelines across this part of the brain regions this is similar to the anterior-posterior variation observed in the pigeon HA (Mehlhorn et al., 2022). In adults, the low density of DCX-positive cells signifies a significant reduction in neurogenesis and neuronal migration this is consistent with what is obtainable in quail (Nkomozepi et al., 2018, 2019). It should be noted that the reduction does not mean absence because the studies by Mazengenya demonstrated the presence of neurogenesis in the adult quail brain, especially in the telencephalon and optic bulb of the adult quail brain.

This is what is obtainable in other species and reported in many studies (Anat & Pravosudov, 2012; D'Amico et al., 2011; Mehlhorn et al., 2022; Ngwenya et al., 2018). While the reduction reflects the maturation and stabilization of neural circuits the decline in neurogenesis with age aligns with the established knowledge in developmental neurobiology, where early development is marked by high neurogenic activity that diminishes as the brain matures (Owji & Shoja, 2020). This supports the idea that while juvenile and subadult brains exhibit significant plasticity and ongoing development, adult brains show reduced neurogenic processes, focusing on maintaining established neural structures.

The similarly observed DCX in Dorsal and Intermediate Hyperpallium (HD and HI) along with the neuronal migration during the developmental stages are essential for brain development and functional maturation. The migrating neuroblasts confirm the ongoing cell movement necessary for the formation of neural circuits (Accogli et al., 2020). The low density of DCX-positive cells in the HD and HI regions of the adult brain confirms the reported decrease in neurogenesis and migration as the brain matures. This is also consistent with the general trend in brain development across species, where neurogenesis is robust during early stages to support growth and decreases significantly in adulthood as the brain's structure becomes more stable and established (Owji & Shoja, 2020). This probably allows the adult brain to focus on maintaining the existing neural networks.

For Medial Pallium (M) and Mesopallium (Mst), The lateral ventricles are known sites for neurogenic niches in canaries' (Melleu et al., 2013) and pigeon (Ball & Balthazart, 2007) brain, where new neurons are generated and migrate to various brain regions. Therefore, the observed high density of DCX-positive fibres around these areas is not surprising.

This may be because they are crucial for producing and supporting the migration of new neurons. These contribute to the growth and development of neural circuits in the pallium. As the density decreases towards the back of the brain, it indicates a rostral-caudal gradient in neurogenesis and migration.

This may be due to brain's developmental timeline where anterior regions typically mature earlier than posterior regions. The results also reflect a significant decline in adult as the brain matures and stabilizes its neural networks. For the NI region, the results can be translatable to a limited neurogenesis and neuronal migration compared to other brain areas during these developmental stages. The absence of DCX-positive cells in the core region suggests that the neurogenic activity is either minimal or absent in this central part of the NI contrary to what is obtainable in the pigeon brain (Mehlhorn et al., 2022). The anterior commissure (CA) connects the two hemispheres of the brain. The low density of DCX-positive cells around the CA might reflect a more specialized and less plastic role of the NI in the developing quail brain, possibly focusing on the stabilization of existing neural connections rather than the generation of new neurons.

The lack of DCX-positive cells in the entopallium, globus pallidus, and arcopallium across all stages of development. This agrees with the previous research (Nkomozepi et al., 2019) and revealed the importance of neuronal stability in these regions probably because their role may be compromised by ongoing neurogenesis.

These observations are consistent with past literature, where regions involved in fundamental and highly specialized functions often exhibit low or no post-developmental neurogenesis to maintain functional stability (Kempermann, 2022). This pattern reflects a balance in the brain between areas that continue to adapt and grow and those that require long-term consistency for optimal function.

For the hippocampal formation, the lowest density of DCX-positive cells during the juvenile and subadult stages also indicates minimal neurogenesis and neuronal migration compared to other brain regions. This suggests that these areas rely on early established neurons for their roles in learning, memory, and spatial navigation. The moderate density of DCX-positive cells which lack processes indicates that while some neurogenesis persists, the new neurons may not fully mature or integrate into the neural circuits. This is not surprising considering the functional importance of these regions.

For the CDL and CPi the observed distribution indicates ongoing neurogenesis and neuronal migration throughout development in these regions. The persistent high density of DCX-positive cells suggests that CDL and CPi are actively involved in generating new neurons and incorporating them into the existing neural circuits across various developmental stages. Similar finding were also observed in ostrich and emu (Mazengenya et al., 2020) the Dorsolateral Cortical Area and Piriform Cortex are implicated in sensory processing, olfaction, and memory formation (Atoji & Wild, 2006). The continual neurogenic activity observed in these regions may contribute to their plasticity and adaptability, allowing for the continuous integration of new information and experiences throughout the lifespan of the quail. This pattern of high neurogenic activity revealed the importance of CDL and CPi in the dynamic processes of brain development and function, emphasizing their roles in supporting sensory perception and learning abilities in the quail. The observed high to moderate in the striatum revealed the growth and the formation of new neural connections essential for the reward learning and decision-making functions associated with the striatum (Cox & Witten, 2019).

In the adult stage, the noticeable decrease in the density of DCX-positive cells and the presence of migrating neuroblasts suggests that; while neurogenesis slows down in adulthood, there remains some ongoing production and migration of new neurons. This indicates a continued but reduced capacity for neural plasticity and repair within the Striatum (Lovinger, 2010), which may be crucial for its functional role. The observed decrease in neurogenesis aligns with the typical developmental trend where brain regions establish their primary neural circuits during early stages and then focus on maintaining and refining these circuits as the organism matures.

In the diencephalon, low DCX-positive cells were observed in the Medial hypothalamus, adjacent to the subventricular zone (SVZ) of the third ventricle in the brain during the juvenile stage. These suggests that there was some level of neurogenesis and neuronal migration in this region early in development aligning with studies in the rodents (Stocks, 2016).

However, in the subadult and adult stages, the absence of DCX-positive cells indicates the significant decline or ceasing of neurogenic activity in the medial hypothalamus as the quail matures. The earlier might be related to the developmental processes required for establishing the hypothalamic functions, which include regulation of endocrine activities, homeostasis, and various autonomic processes and the later suggests that the medial hypothalamus achieves its mature structure and function early on, relying on the established neurons for its regulatory roles throughout the rest of the lifespan (Pop et al., 2018). This is consistent with the broader understanding of hypothalamic development, where early neurogenesis is crucial for setting up the necessary neural circuits, followed by a maintenance phase with absence of minimal neuron production in adulthood.

In the Dorsolateral thalamic nuclei (DMA, DLL, DLM) of the quail brain, DCX-positive cells were present during the juvenile stage, indicating active neurogenesis and neuronal migration in these regions early in development. This activity is crucial for the initial formation and maturation of thalamic circuits, which play key roles in sensory processing, motor control, and the relay of information between different brain regions (Sherman, 2007). However, in the subadult and adult stages, there are no DCX-positive cells observed in the nuclei. This absence suggests that neurogenesis and neuronal migration significantly decrease or cease as the quail matures. The early presence of DCX-positive cells in the dorsolateral thalamic nuclei aligns with the need for these areas to establish their functional neural networks during development, ensuring proper sensory and motor integration. The lack of DCX-positive cells in later stages indicates that the thalamic circuits become stable and rely on the maintenance and optimization of existing neurons rather than the incorporation of new ones. This developmental pattern is consistent with the thalamus's role in providing a stable and efficient relay system essential for processing and transmitting neural information throughout the brain.

The consistent absence of DCX-positive cells in both the mesencephalon and optic tectum across all ages revealed the need for stability probably due to their roles require that the neural circuits remain stable and reliable. This developmental pattern aligns with the broader understanding that certain brain regions require early establishment and maintenance of their neural architecture to support their specialized and critical functions effectively (Culig et al., 2022; Olaleye & Ihunwo, 2014; Owji & Shoja, 2020). The lack of neurogenesis ensures that these areas can provide consistent and uninterrupted capabilities essential for the bird's interaction with its environment.

The cerebellum is responsible for balance, and motor coordination, and the Purkinje cells are central to its function. During the juvenile and subadult stages, the high density of DCX-positive fibres supports the development and refinement of these motor functions, allowing the quail to acquire and perfect movements and motor skills. The low density of DCX staining in adults reflects a transition to a more stable and maintained state, where the established neural circuits are optimized for consistent performance. This developmental pattern aligns with the broader understanding that the cerebellum requires early neurogenesis to establish its functions but relies on the stability of these circuits in adulthood for precise motor control and this pattern is highly conserved among the amniotes (Rueda-Alaña & García-Moreno, 2022).

5.8 Double labelling immunofluorescence of neural stem, glia and neurons

Neural Stem and progenitor cells (SOX2 and GFAP expression)

The co-localization of SOX2 and GFAP in the ventricular zones, hippocampus, and pial surface demonstrates the persistence of neural stem and progenitor populations in the quail brain throughout life. SOX2 is a transcription factor critical for maintaining stemness and self-renewal in neural progenitor cells, while GFAP marks radial glia-like stem cells and mature astroglia (Komitova & Eriksson, 2004; Migaud et al., 2010). The presence of SOX2+/GFAP+ cells with radial fibers extending toward the pial surface in this study supports their identity as active radial glial stem cells, consistent with reports from other avian species such as the canary and zebra finch (Alvarez-Buylla et al., 1990; Vellema et al., 2010).

In the Japanese quail, these cells were especially abundant in the ventricular zones along the anterior–posterior axis, as well as in the hippocampus and optic tectum. These regions correspond to known neurogenic zones in birds, where radial glia persist and generate new neurons throughout life (Barnea & Nottebohm, 1994; Melleu et al., 2013). This pattern parallels mammalian neurogenesis, where GFAP+/SOX2+ radial glia persist in the subventricular zone (SVZ) and subgranular zone (SGZ) of the hippocampal dentate gyrus (Doetsch et al., 1999; Seri et al., 2001).

Thus, the high density of SOX2-positive cells in the ventricular zones, pial surface, optic tectum and hippocampal formation of the quail brain suggests the presence of multiple, active neurogenic niches like those described in mammals, supporting the notion that the avian brain retains robust neurogenic capacity throughout life.

Astrocytic differentiation (GFAP and S100A1 expression)

The co-expression of GFAP and S100A1 observed in the mesencephalon and brainstem indicates active astrocytic differentiation and maturation. GFAP is a marker for both radial glia and mature astrocytes, while S100A1, a calcium-binding protein, identifies differentiated astrocytes with roles in calcium homeostasis, synaptic regulation, and metabolic support (Donato et al., 2013). In the present study, S100A1+ cells displayed two distinct morphologies which are the stellate and elongated indicating astrocytic heterogeneity, a feature that has been described in the chicken and pigeon brain (García-Moreno et al., 2018). The GFAP+/S100A1+ co-labelling confirms the progression from progenitor to mature astrocyte stages, signifying ongoing astrogliogenesis. These astrocytes likely play essential roles in neuronal protection, maintenance, and repair, similar to those in mammalian systems (Molofsky & Deneen, 2015).

The presence of these astrocytes across multiple brain regions suggests that gliogenesis is widespread in the quail brain and continues throughout life, contributing to its pronounced neural plasticity.

Oligodendroglial differentiation (GFAP and OLIG2 expression)

The observation of GFAP+/OLIG2+ cells in white matter regions such as the optic tract, commissures, and hippocampal areas indicates ongoing oligodendrogenesis in the quail brain. OLIG2 is a transcription factor that regulates the specification and differentiation of oligodendrocytes from glial progenitor cells (Takebayashi et al., 2002). The co-expression of OLIG2 with GFAP suggests a transitional population of glial precursors, bridging radial glia and oligodendrocyte precursor cells (OPCs).

This pattern corresponds to findings in other avian species, such as chicken and pigeon, where OLIG2+ cells are abundant in myelinated tracts and periventricular areas (Tanaka et al., 2007; Kondo et al., 2015). The detection of such cells in adult quail suggests that oligodendrocyte generation and myelin remodeling are ongoing processes, as previously observed in songbirds, where myelination is dynamically regulated during song learning and seasonal changes (Mello et al., 2019).

Comparatively, in mammals, OLIG2+ progenitors arise from GFAP+ radial glia in the SVZ and migrate to white matter to form myelinating oligodendrocytes (Rowitch & Kriegstein, 2010). Therefore, the quail model confirms that the molecular mechanisms of oligodendrogenesis are evolutionarily conserved across vertebrate taxa.

Neuronal differentiation (GFAP and DCX expression)

Doublecortin (DCX) is a microtubule-associated protein expressed in migrating and immature neurons (Brown et al., 2003). The co-labelling of GFAP and DCX across telencephalic and ventricular regions indicates active neuronal differentiation from radial glia-like progenitors. The rostro-caudal distribution of DCX+ cells along the ventricular wall and their widespread presence in the pallium suggest continuous neuronal production and migration, consistent with descriptions in other birds (Melleu et al., 2013; Scott et al., 2012).

The detection of two morphological DCX+ cell types, some with long processes and others rounded and reflects progressive maturation stages, as also observed in the zebra finch and canary (Vellema et al., 2010). In mammals, DCX+ immature neurons are restricted to the hippocampal SGZ and SVZ; thus, their broad distribution in the quail suggests that adult avian neurogenesis is more extensive and regionally diverse, likely underpinning the superior neural plasticity observed in birds (Barnea & Pravosudov, 2011; Clayton, 2016).

Comparative and evolutionary implications

The results demonstrate that the Japanese quail retains active neurogenic and gliogenic potential into adulthood, highlighting its suitability as a model for studying vertebrate neural regeneration. The overlapping expression of SOX2, GFAP, S100A1, OLIG2, and DCX indicates a range of cellular transitions to lineage commitment. These findings are consistent with prior studies in songbirds, where adult neurogenesis contributes to vocal learning and behavioral adaptability (Nottebohm, 2004; Barnea & Pravosudov, 2011) and pigeons, where (christen melhornm et).

Compared to mammals, where neurogenesis is largely restricted to specific regions, birds exhibit multiple neurogenic niches distributed throughout the brain (Wilhelmsson et al., 2012). The quail thus represents an evolutionary intermediate model, exhibiting both avian-specific feature, such as persistent radial glia and mammalian-like lineage segregation, making it ideal for comparative studies of brain plasticity, repair, and aging.

Collectively, these findings establish the Japanese quail as a robust avian model for understanding the molecular and cellular dynamics of neurogenesis and gliogenesis, with significant relevance to regenerative neuroscience and comparative brain evolution.

5.9 Ultrastructural study of neuron, glia and mitochondria in the quail brain

The avian brain shows significant plasticity and complexity, making it a useful model for studying neurodevelopment and gliogenesis (Kirkwood, 1998, 1999). The Japanese quail (Coturnix japonica) is an established model organism in neurobiological research owing to its easily identifiable and accessible growth stages that allow for experimental studies (Cheviron *et al*., 2014; Olkowicz *et al*., 2016). High-resolution imaging has also been achieved via transmission electron microscopy (TEM) (Gupta *et al*., 2020) to examine glial and neuronal structures, revealing cellular morphology and subcellular elements such as mitochondria. Building on this foundation, our study aimed to expand our understanding of avian brain ultrastructure The study investigated the ultrastructural features and localization of glial cells, neurons, and mitochondria in the pallium, mesencephalon, optic tectum, and cerebellum of juvenile, subadult, and adult male Japanese quail using electron microscopy.

Electron microscopy analysis revealed several key observations across different brain regions and developmental stages. These findings revealed a rich presence of microglia, oligodendrocytes, and mitochondria in the pallium and cerebellum of the juvenile brain, with the hippocampus exhibiting a particularly high concentration of mitochondria. The elevated mitochondrial density in the hippocampus suggests an increased energy demand, likely supporting the intense neuronal activity and plasticity associated with its function in memory formation and spatial navigation. Mitochondria in these brain regions display diverse morphologies, ranging from round to elongated, with normal cristae structures, indicating healthy and functional organelles that are capable of efficient energy production.

The presence of elongated mitochondria encased in myelin sheaths suggests a close association between energy-producing organelles and myelination, facilitating high-energy requirements of oligodendrocytes during myelin formation and maintenance.

Neurons exhibited enlarged nuclei with centrally located dark-stained nucleoli, dark aggregates, or clumped granules in the nuclei. The Nissl bodies are in contact with the nuclear membrane and proximal dendrites.

These ultrastructural observations provided valuable insights into the diverse roles of different cell types in the avian brain. Astrocytes possess thin, irregularly shaped cytoplasm with electron-dense, darkly stained inclusions and potato-shaped nuclei.

Oligodendrocytes, however, have a thin, irregularly shaped, and darkly stained cytoplasm, with round or oval shaped. The nuclei were darkly stained and contained multiple heterochromatin clumps. Microglia exhibit cytoplasmic features similar to oligodendrocytes, with irregular nuclei containing more heterochromatin granules, forming a grid across the nucleus.

These findings provide insight into the ultrastructural organization and developmental changes in the Japanese quail brain, highlighting the importance of glial cells, neurons, and mitochondria in supporting brain function and development. Neurons act as the main functional components of the nervous system, whereas glial cells fulfill essential supportive functions.

Astrocytes, oligodendrocytes, and microglia are glial cells that are crucial for the maintenance of brain homeostasis, support of neuronal function, and myelination (Verkhratsky *et al*., 2016). In contrast, neurons are the principal functional units of the nervous system; they rely almost exclusively on the mitochondria to meet ATP requirements and bind calcium.

The distribution and morphology of mitochondria in these neuronal structures reflect varying energy demands across brain regions. The morphology and distribution of mitochondria mirror the metabolic requirements of distinct brain regions. The long mitochondria of the telencephalon and the round mitochondria of the cerebellum may adapt to different levels of energy demand (Devine & Kittler, 2018). Indeed, the apparent localization of mitochondria in astrocytes close to blood vessels supports their function in metabolic coupling between neurons and vasculature (Magistretti & Allaman, 2018).

The gradual increase in mitochondrial density and morphological complexity recorded in this study corresponds to observations in other avian taxa (Olkowicz *et al*., 2016; Stacho *et al*., 2020).

Mitochondrial traits in quails change as they develop, reflecting broader transformations in brain structure and function. The longer mitochondria in neurons indicate increased oxidative metabolism, which is favorable for synaptic function and plasticity (Gupta *et al*., 2020).

Almost all transcripts of gliogenesis have increased expression during development, with astrocytes showing enlarged and complex cytoplasm and oligodendrocytes producing functionally mature myelin sheaths (Marques *et al*., 2016). These results provide valuable insights into avian neurobiology and highlight the utility of ultrastructural studies in understanding cellular and subcellular organization. Collectively, these ultrastructural observations offer a detailed perspective on the cellular and subcellular arrangement within the brain of the Japanese quail.

In conclusion, the insights gained from examining the ultrastructural neuroanatomy of glial and neuronal cells in the brain of Japanese quail enhance our understanding of the shape and placement of mitochondria.

CHAPTER SIX: Summary, conclusions and recommendations

6.1 Highlights of the study

This study uniquely integrates histology (Nissl, myelin staining), immunohistochemistry (SOX2, PCNA, GFAP, S100A1, OLIG2, IBA-1, DCX), double-labeling immunofluorescence, TEM to reveal gliogenesis patterns in the male Japanese quail brain, showing stage-specific glial development and regional specialization. Understanding gliogenesis in the quail brain is crucial for unraveling how glial cells shape neural development and function in birds. My PhD thesis filled a gap in avian neuroanatomy, offering a multi-method approach that can inform broader neuroscience research. It highlights the quail as a valuable model for studying brain maturation, with potential applications in developmental biology and glial-related disorders

6.2 Summary of the study

My PhD thesis explored gliogenesis in the male Japanese quail brain through five complementary approaches across separate results sections in chapter four. Chapter 4.1 used Nissl and myelin silver staining to map the histological structure of brain regions, identifying glia and neuron and myelin distribution for myelinated fibers in juvenile, subadult, and adult stages. It also looked at the statistical significance in body and brain weight across the experimental groups. Chapter 4.2 employed immunohistochemistry with markers SOX2 (neural stem cells), PCNA (cell proliferation), GFAP and S100A1 protein (astrocytes), OLIG2 (oligodendrocytes), IBA-1 (microglia), and DCX (immature neurons).

Chapter 4.3 dealt with double-labeling immunofluorescence via confocal laser scanning microscope, to track glial development and cellular dynamics across the different age stages. Chapter 4.4 utilized transmission electron microscope (TEM) to examine the ultrastructure of glial and neuronal cells, revealing fine details of organelles and cellular interactions and the morphology of mitochondria. Together, these sub chapters demonstrate that gliogenesis in the quail brain follows distinct temporal and spatial patterns, with glial subtypes emerging in a region-specific way, influenced by developmental stage.

6.3 Study significance

This research provides the first detailed analysis of gliogenesis in the male Japanese quail brain across multiple developmental stages and techniques. By combining histology, IHC, and TEM, it offers a robust dataset on glial cell formation, distribution, and structure in an avian model. The findings enhance our understanding of brain development beyond mammalian systems, contributing to comparative neurobiology and the role of glia in neural health.

6.4 Limitations of the study

Although the study documents gliogenesis and neurogenesis in the quail brain, certain limitations were encountered during the study.

a. Most of the stains worked and managed to characterize the cells. Glia markers, especially IBA-1, did not fully characterize the microglia. OLIG2 and GFAP from abcam did not work on the quail brain at all.
b. The current study was designed to determine age related differences in the expression of the selected (SOX2, PCNA, GFAP, OLIG2, IBA-1and DCX) and to greater extent achieve this goal.

c. The study design requires the ultrastructure of glia, neuron and mitochondria in all brain regions, but due to corvid-19 endemic that led to the closure of the microscopic and microanalysis unit limited the TEM study to only five brain regions.
d. The study was conducted only on male Japanese quails. However, the study could not compare between male and females animals since the females were excluded from the original study design.

6.5 Conclusions

This study concludes that gliogenesis in the male Japanese quail brain is a dynamic process varying by region and developmental stage. Histology showed progressive structural organization, with increased myelination in adulthood. IHC revealed active glial proliferation in juveniles, peaking in subadults, and stabilizing in adults, with astrocytes and oligodendrocytes dominating distinct areas. TEM confirmed ultrastructural maturation, including organelle development in glial cells. These results suggest that gliogenesis supports neural development and function in a coordinated, stage-specific manner in the quail brain.

6.6 Future scope

Future studies could explore gliogenesis in female quails to assess sex differences, investigate hormonal influences or extend the analysis to other avian species. Additional molecular techniques, such as RNA sequencing, could uncover gene networks driving gliogenesis. Functional studies linking glial changes to behavior or disease models in quail could also expand the research's impact. Quantification will enhance the understanding of the extent of AHN and gliogenesis in various age groups.

This helps to identify the age stage with the maximum numbers of news glia and or neurons which will be ideal for model animal selection and form basis for prediction of treatment outcomes in individuals of various ages. Comparative study with a different taxon of birds like (parrots, crow and canary) and mammals.

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APPENDIX

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2018/11/59/A

APPLICANT: Mr A Muhammad

SCHOOL: Anatomical Sciences
DEPARTMENT:
LOCATION:

PROJECT TITLE: Gliogenesis in the Male Japanese Quail (Curtonix Japonica) Brain

Number and Species

30X 3-12 weeks old male Curtonix Japonica Japanese quail

Approval was given for the use of animals for the project described above at an AESC meeting held on 2018/11/27. This approval remains valid until 2021/01/15.
Unreported changes to the application may invalidate the clearance given by the AESC
An annual progress report must be provided

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

Signed: _____ (Chairperson, AESC) Date: 22/01/2019

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 16 January 2019

cc: Supervisor: Prof A Ihunwo and Dr P Mazengenya
Director: CAS

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