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# The presence and pattern of adult neurogenesis in the brains of three prosimian primates.

**By:**

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A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand in fulfilment of the requirements for the degree of Master of Science in Medicine.

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**DECLARATION**

I,Thandi Mamorapelo Dorothy Fasemore, hereby declare that this thesis is my own work. It is being submitted for the degree of Master of Science in Medicine (Neurosciences) in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university

Signature……

Date……

**DEDICATION**

*To the Human heart that struggles to find the joy, fulfilment and satisfaction in life through God's presence. By Dr Myles Munroe*

To my heavenly Father, God Almighty for giving me the strength to cope, ability to push against all odds and most of all for giving me the wisdom not to quit when I was facing the storms of life. Thank for your presence as it assured me that it is well with me.

To my mother Tshatiwe Nqinileyo thank you for being a pillar of strength and always expressing your desire for your children to get educated. Thank you for always being there to step in when helpers left me stranded with my children. To my husband Dr Olufemi Fasemore thank you for your support and understanding. Most of all thank you for your sacrifice and being the pillar and the backbone of our family. To my two sons Olulana and Ayomide Fasemore even though you never understood why mommy has to drop you early at school and pick you late after all children have gone home, your presence always made me to have joy and strive to do better in life. I thank God for giving me you.

## PUBLICATIONS AND PRESENTATIONS ARISING FROM THE STUDY

A poster presented at the Joint Wits and German summer school which was held in the school of anatomical sciences on the 18th -22nd February 2013.

Abstract has been submitted and selected for a poster presentation at the 25th ISN-APSN Joint Biennial meeting in conjunction with ANS which was held on the 23rd – 27th August 2015, Cairns, Australia.

**ABSTRACT**

This study investigated the presence and pattern of adult neurogenesis in the Subventricular zone (SVZ) of the lateral ventricle, the dentate gyrus (DG) of hippocampus and potential neurogenic sites in three prosimian primates. While two nocturnal species, the *Galagoides demidoff phasma* (Galago) and the *Perodictus potto* (Potto) were caught in the wild, the *Lemur catta* (Lemur) was a zoo kept diurnal animal. Two brain specimens from each species, perfusion-fixed with 4% paraformaldehyde were cut at 50 μm thick frozen sections in sagittal and coronal planes. Using doublecortin (DCX) and Ki-67 antibodies, immature neurons and proliferating cells were identified respectively in the SVZ and DG and in potential sites such as the striatum, corpus callosum, amygdala, and piriform cortex in all the three species. DCX positive cells were observed in the cerebellum of the Lemur and the Galago but not in the Potto. There were no Ki-67 proliferating cells observed in the cerebellum and the neocortex of all the three species. Interspecies analysis indicated that the estimated rate of Ki-67 proliferating cells in Potto was 1.9 times higher than that of the Lemur and 4.8 times higher than that of the Galago. There was no statistical significant variation in the number of estimated Ki-67 cells within the three species but a significant difference ($P \leq 0.05$) when comparing Potto with the Lemur and Galago. There was no significant difference ($P \geq 0.05$) in the number of Ki-67 cells between the Lemur and the Galago. Variations do exist in the cell proliferation pattern among these three prosimian primates.

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**NOMENCLATURE**

III = Oculomotor nucleus

IV = Trochlear nucleus

Vmot = motor division of trigeminal nerve nucleus

VI = Abducens nucleus

VIId = Dorsal division of facial nerve nucleus

VIIv = Ventral division of facial nerve nucleus

X = Dorsal motor division of vagus nucleus

XII = Hypoglossal nucleus

3N = Oculomotor nerve

M1 = Primary motor cortex

V1 = Primary visual cortex

ABC = Avidin-biotin complex

BB = Blocking buffer

BDNF = Brain derived neurotrophic factor

BrdU = Bromodeoxyuridine

CA 3 = Cornu Ammonis 1-3

CNS = Central nervous system

DAB = 3, 3´-diaminobenzidineV2 = Secondary Visual cortex

ac = Anterior Commissure

Amyg = Amygdala

C = Caudate

cc = Corpus callosum

Cer = Cerebellum

Cl = Claustrum

DCN = Deep cerebellar nuclei

DCX= Doublecortin

DG = Dentate Gyrus

DT = Dorsal thalamus

f = Fornix

GC = Central gray matter of the midbrain

GP = Globus pallidus

Gr = Gracile nucleus

Hb = Habenular

Hip = Hippocampus

Hyp = Hypothalamus

ic = Internal capsule

IC = Inferior colliculus

ICP = Inferior cerebellar peduncle

Io = inferior olivary nucleus

Lc = Locus coerulus

Lgd = dorsal lateral geniculate nucleus

Lgv = ventral lateral geniculate nucleus

LV = lateral ventricle

MB = Mammilary bodies

Mc = main cluster of orexinergic neurons

mcp = Middle cerebellar peduncle

Neo = Cerebral Neo-Cortex

N.Acc = Nucleus Accumbens

N.Amb = Nucleus Ambiguus

OB = Olfactory Bulb

OC = Optic chiasma

ON = Optic nerve

OT = Optic tract

OV = Olfactory ventricle

P = Putamen nucleus

Pir = Piriform Cortex

PSA-NCAM = Polysialyted neural cell adhesion molecule

PTa = Pretectal area

R = reticular nucleus of the dorsal thalamus

Rmc = magnocellular division of red nucleus

RMS = Rostral Migratory Stream

S = Septal nuclear complex

SC = Superior colliculus

SCN = Suprachiasmatic nucleus

SCP = Superior cerebellar peduncle

TOL = Olfactory tubercle

Str = Striatum

SVZ = Subventricular zone

TMS = Temporal Migratory Stream

VPO = Ventral pontine nucleus

WM = white matter of the cerebral cortex

Zi = Zona incerta

# CHAPTER 1

## 1.1 Introduction

Neurogenesis is the process by which neurons are generated. It is most active during pre-natal development and the rate slows down during postnatal development. Adult neurogenesis is the process by which the adult neural stem cells or a precursor cells divide into different types of cells through a multistep process that involves cellular events such as proliferation, differentiation, migration, targeting and synaptic integration of neurons (Kempermann, 2004; von Bohlen *et al.*, 2007).The stem cells are natural cells that can either be totipotent (a cell that develop into a new individual), pluripotent (a cell that can form any cell types) or multipotent (differentiated cells that can form several other tissues) in nature (Ihunwo, 2011).

Two regions in the mammalian brain have been established to be the active neurogenic sites, the subventricular zone (SVZ) lining the lateral ventricles, and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Amrein *et al.*, 2007; von Bohlen *et al.*, 2007; Ihunwo and Pillay, 2007, Patzke *et al.*, 2015). The rostral migratory stream (RMS) is the major pathway by which progenitor cells migrate from the subventricular zone (SVZ) to the olfactory bulb (OB). The SVZ neurogenesis plays a role in defending the viral spread from the central olfactory pathway, repair injuries and also involved in the fine modulation of olfaction (Gheusi and Lledo, 2014; Yuan and Slotnick, 2014).This pathway of cells was reported to be present in rodents (Craig *et al.*, 1999; Lois and Alvarez-Buylla, 1994), rabbits (Fasolo *et al.*, 2002; Ponti *et al.*, 2006), squirrel monkeys (Bedard *et al.*, 2002), rhesus monkeys (Pencea *et al.*, 2001) and in humans (Eriksson *et al.*, 1998; Kam *et al.*, 2008).

The hippocampal neurogenesis is reported to be important in hippocampus dependent cognitive functions (Sahay *et al.*, 2011; Patzke *et al.*, 2013; Patzke *et al.*, 2015). Adult neurogenesis in the DG is a process that involves many stages that can be defined as the cell

proliferation, migration, differentiation and ultimately the maturation of granule cells, and these new neurons are generated in the subgranular zone of the dentate gyrus and then migrate into the existing granule cell layer (von Bohlen *et al.*, 2007; Kempermann, 2012; Patzke *et al.*, 2015) where they integrate into the existing circuitry (Lie *et al.*, 2004). A dividing progenitor cell gives rise to daughter cells which differentiate, migrate, and integrate extending dendrites towards the molecular layer and an axon towards the CA3 region of the hippocampus (Kempermann *et al.*, 2004).

Other reported potential neurogenic sites are the cerebral cortex (Gould *et al.*,1999b; Gould and Gross, 2002), the ependymal layer of the third ventricle ( Xu *et al.*, 2005), olfactory bulb and tubercle (Berdard *et al.*, 2002;), the sub-ependymal layer (Peretto *et al.*, 1997) the amygdala (Bernier *et al.*, 2002; Fowler *et al.*, 2005), the substantia nigra (Zhao *et al.*, 2003), the septum striatum (Dayer *et al.*, 2005; Berdard *et al.*, 2006 and Luzzatti *et al.*, 2006), the brainstem (Bauer *et al.*,2005), the hypothalamus (Kokoeva *et al.*, 2005), the corpus callosum (Kaplan, 1977; Gage and Van Praag, 2002; Gould and Gross, 2002) as well as the piriform cortex (Pekcec *et al.*, 2006, Nacher and Bonfati, 2002) and the Cerebellum (Ponti *et al.*, 2006; 2008).

## CHAPTER 2

### 2.1. Literature review

#### 2.1.1 History of Neurogenesis

The breakthrough on adult neurogenesis came about in the late 1950s with the introduction of [$^3$H] thymidine autoradiography, whereby this method was the first to be used in studying cell proliferation in the adult brain (Gross, 2000). Altman was reported to be among the first scientists who reported evidence for new neurons in the neocortex, dentate gyrus and olfactory bulb of young and adult rats using the [$^3$H] thymidine autoradiography method (Gross, 2000 ; Gould, 2002). Altman's findings were ignored or dismissed, because one of the issues that brought about doubt concerning his findings was the uncertainty of whether the adult generated cells were neurons or cells of glial origin.

Kaplan (1977) examined the [$^3$H] thymidine labeled cells in the cerebral cortex, hippocampus and olfactory bulb of adult rats and confirmed that they were newly generated neurons. [$^3$H] thymidine is normally taken up by the cells undergoing DNA synthesis in preparation for mitosis; hence it was used as a marker for proliferating cells (Gould and Gross, 2002). The 5'-Bromo-2-deoxyuridine (BrdU) is a thymidine analog that can be incorporated into the DNA only during the S-phase of the mitotic process, thus the substitution of an endogenous base [$^3$H] thymidine, with BrdU enabled researchers to label specific proliferating cells and estimate the number of new cells produced (Kee *et al.*, 2002). BrdU was administered on cancer patients as part of their treatment, and it was confirmed that positive BrdU cells were identified in the post-mortem tissue sample and that the surviving cells were neurons (Erikson *et al.*, 1998).

Despite its advantages the [$^3$H] thymidine autoradiography, its wide use in several studies showed some limitations and pitfalls on its use. BrdU administration involve the following

steps, it has to be injected intraperitoneally into the experimental animal, followed by a survival time for tracking the dividing cells before immunohistochemical detection methods are done, however this method was reported to produce some toxic effects especially if administered in high dose (Kee *et al.*, 2002; Koketsu *et al.*, 2003). Also since BrdU is not a marker of cell proliferation but a marker of DNA synthesis (Taupin, 2007), therefore it can have adverse effects on the study and analysis of adult neurogenesis if proper measurements and controls are not taken into consideration thus false results can be reported. Numerous side effects of BrdU were reported by Taupin (2007), and some of these were that it triggers cell death, the formation of teratomas, alters DNA stability, prolongs cell cycle and has translocational and translational effects on cells that incorporate it.

Several studies also reported that BrdU has the possibility of producing mutated cells, thus the consequence of this was the severe abnormalities in the developing tissue (Kolb *et al.*, 1999; Koketsu *et al.*, 2003; Taupin, 2007). There were also reports about problems in diffusion of BrdU in various body tissues following intraperitoneal injection (Kee *et al.*, 2002). Cameron and Mckay (2001) reported that the blood brain barrier prevented diffusion of BrdU into the brain tissue in old animals. It was also reported to increase the adrenal glucocorticoid secretion, which have suppressive effect on cell proliferation in the dentate gyrus (Gould and Tanapat, 1999). Despite all these side effects the administration of BrdU before and after experiment itself exposes an animal to some level of stress, which can alter incorporation of the dividing neurons by stimulating hippocampal glutamate release which then will naturally inhibit cell proliferation (Gould and Tanapat, 1999). The administration of BrdU is not only stressful but also a time consuming process which led to a development of new strategies to study cell proliferation and neurogenesis.

### 2.1.2. Factors affecting Adult Neurogenesis

Adult neurogenesis has been reported to be affected by a variety of factors. These factors can be genetical, environmental and pathological (Ihunwo, 2011) and can either increase or decrease the rate of adult neurogenesis. Some of the environmental factors reported to affect the rate of adult neurogenesis are stress (Gould *et al.*, 1997), and exercise ( Van Praag *et al.*, 1999). Stress is reported to generally inhibit proliferation of new cells and thereby decreases neurogenesis in the hippocampus (Cameron *et al.*, 1994; Gould *et al.*, 1997). Chronic stress has been reported to result in persistent inhibition of granule cell production and changes in the structure of DG (Gould *et al.*, 1999), therefore the longer the animal stay in a stressful condition the greater the rate of granule cell inhibition.

Studies by Tanapat and colleagues (2001) demonstrated that in rodents that were exposed to predator odour stress, there was a decreased cell proliferation and that the repeated stress results in atrophy of CA3 pyramydal neurons. Also a study by Czeh and colleagues (2007) demonstrated that chronic stress alters dendritic morphology in the hippocampus and the medial prefontal cortex, whereby the mature principal neurons were reported to retract their dendritic tree and receive reduced number of synaptic contacts. In contrast to these findings a study of rodents by Parihar and colleagues (2009) suggested that coping with mild intermittent stress increases adult neurogenesis in the hippocampal dentate gyrus. Confirmatory to the above study a review study by Lyons and colleagues (2010) revealed that intermittent stress exposure in rhesus monkeys increased hippocampal neurogenesis. Also Marlatt and colleagues (2011) exposed the common marmoset monkeys to psychosocial stress for a period of 2 weeks and found that it failed to alter the number of BrdU positive and DCX positive cells both in the hippocampus and the amygdala.

The pathological factors such as brain ischemic injuries (Koketsu *et al.*, 2006; Tonchev and Yamashima, 2006; and Gould, 2007), chronic epileptic seizures (Hattiangady *et al.*, 2004; Park *et al.*, 2006) and inflammation (Ekdahl *et al.*, 2003) down regulate adult neurogenesis whereas diseases such as diabetes, stroke, Parkinson's disease and Alzheimer's disease are known to increase the rate of neurogenesis (Danzer, 2008). The process of aging is also reported to affect the rate of adult neurogenesis (Kuhn *et al.*, 1996; Gould *et al.*, 1999a; Aizaiwa *et al.*, 2009). A study by Lupien and Colleagues (1994) reported that aging is associated with increased levels of cortisol thus this will lead to decreased rate of neurogenesis in older animals.

Kempermann and Gage (2002) reported that variations in the rate of adult neurogenesis in the brains of different strains of mice were due to a possible genetic role. Other factors such as environmental enrichment (Kempermann *et al.*, 1997; Ehninger and Kempermann, 2003), and learning (Gould *et al.*, 1999) and social interaction increase the rate of neurogenesis (Boonstra *et al.*, 2001). Lyons and colleagues (2010) discovered that spatial learning was enhanced in conditions that increased hippocampal neurogenesis in rhesus monkeys. Lyons and colleagues (2010) further reported that the corresponding changes were discerned in the expression of genes involved in hippocampal neurogenesis and learned aspects of behaviour change.

Other factors reported to affect the rate of adult neurogenesis are chemical factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF) (Bonfanti and Ponti, 2008), peptide hormones and glucocorticoids (Gould *et al.*, 1992). Oestrogen (Brännvall *et al.*, 2002; Perez-Martin *et al.*, 2003), antidepressant drugs (Malberg *et al.*, 2000; Manev *et al.*, 2001), growth factors such as brain-derived neurotrophic factor (BDNF) (Zigova *et al.*, 1998) and insulin growth factor 1 (IGF-1) (Aberg *et al.*, 2000), were reported to up-regulate neurogenesis.

## 2.2. EVIDENCE OF ADULT NEUROGENESIS

### 2.2.1. ESTABLISHED ACTIVE NEUROGENIC SITES

**Subventricular zone of the Lateral ventricles**

The newly generated cells in the SVZ of the lateral ventricle migrate through the rostral migratory stream to the olfactory bulb and other potential sites of the brain. These cells migrate by chain migration using each other as the migratory substrate (Taupin, 2007). The following primate studies have confirmed the existence of neurogenesis in the SVZ; Gould *et al.*, (1997) in treeshrews; Gould *et al.*, (1999a) and Kornack and Rakic (1999) in macaques (*Macaca mulatta and Macaca Fascicularis*), [Gould *et al.*, (1998); Sawamoto *et al.*, (2011) and Marlatt *et al.*, (2011)] in Marmoset monkeys. In humans numerous numbers of cells that were showing a neuronal phenotype were also observed in the SVZ and the dentate gyrus of the Hippocampus (Eriksson *et al.*, 1998; Ernst *et al.*, 2014).

**Olfactory bulb and Rostral Migratory Stream**

Newly generated neurons migrate through the rostral migratory stream (RMS) to the olfactory bulb and when they reach the olfactory bulb differentiate into interneurons, granule and periglomerular neurons (Taupin, 2007). Despite the marked reduction of olfactory structures in primates, the fate of newly generated SVZ neurons to the OB has been reported to be robust in rodents and non - human primates. Bernier and Parent (1998a, b) detected evidence of adult neurogenesis in the SVZ via the RMS to the olfactory system and more extended to the olfactory tubercle in the adult squirrel monkeys (*Saimiri sciureus*). Other studies of old world monkeys confirmed the migration of newly generated SVZ neurons along the RMS to the OB (Kornack and Rakic, 2001; Pencea *et al.*, 2001; Gil-Perotin *et al.*, 2009; and Wang *et al.*, 2011). Bedard and Parent (2004) reported that the human olfactory bulb is a site of active cell proliferation as well as a recipient of migrating neuroblasts,

however the origin of the cells needed to be determined. Taupin (2006) suggested that the neuroblasts might be traveling in a different path that is yet to be identified. Sanai and colleagues (2004) initially found no migrating neuroblasts from the SVZ along the RMS to the OB of human tissue obtained from the neurosurgical resections when using a marker of cell cycle like Ki-67. Thus the authors concluded that neurons in the adult OB are either not replaced or the precursor might be migrating as individual cells rather than migrating chains. However Sanai and colleagues (2011) later did a comparative study of human brains from infancy to aged adults, and reported neurogenesis in the OB, and that these cells were immature neurons which were migrating through the RMS to the OB and are only limited to infancy period and they become diminished in the adults.

Another study by Bergmann and colleagues (2012) established that there was limited neurogenesis in the olfactory bulb of humans. Despite having neurogenesis in olfactory system in humans (Bedard *et al.*, 2004), it is generally believed that there is no RMS, instead human infants exhibit a different stream known as the medial migratory stream ( Sanai *et al.*, 2011) and that the new neurons generated migrate from the SVZ to the striatum instead of RMS (Ernst *et al.*, 2014). Ernst and colleagues (2014) established through the use of $^{14}C$ dating that in humans majority of the proliferating cells observed in the SVZ migrate to the striatum and few to the OB, which supports the findings of the above studies.

**Temporal Migratory Stream**

The anatomical arrangement of adult forebrain and its extensions is dependent on the species and its cerebral conformation (Bonfati and Ponti, 2008). The postnatal closure of the olfactory ventricle, leaving only the posterior part of the subventricular zone in contact with the ventricle in rodents (Bonfati and Peretto, 2007), has led to them exhibiting a strong migratory pathway, the rostral migratory pathway, thus the absence of temporal migratory stream. In primate species, cells generated in the subventricular zone (SVZ) travel not only

through the rostral migratory stream but also through a unique pathway called the temporal migratory stream (TMS). This cells travel in migratory chains to the amygdala and the entorhinal cortex (Bernier and Parent, 1998 a, b; Marlatt *et al.*, 2011). Bernier and Parent (1998), reported that neurons were produced in the amygdala and piriform cortex and these neurons were adjoining the temporal cortex through a pathway extending from the subventricular zone (SVZ) of the temporal horn to the deep portion of the temporal lobe in New world monkeys (*Saimiri sciureus*) and Old world monkeys (*Macaca Fascicularis*). Marlatt and colleagues (2011) also observed populations of DCX expressing cells in the amygdala of common Marmoset monkeys, and these cells were migrating in chains through the temporal migratory stream to the amygdala and piriform cortex. Also Chawana and colleagues (2013) confirmed neurogenesis in the amygdala and along the temporal migratory stream (TMS) in the Megachiropterans. This pathway is considered to be a common feature of primate neurogenesis.

**The Dentate Gyrus of the Hippocampus**

In the hippocampus, the granule cell precursors are located in the hilus and on the subgranular zone (Gould and Tanapat, 1999). The proliferating granule cell precursors are multipotent and are normally found in the innermost subgranular zone of the dentate gyrus. The newly generated neurons in the SGZ migrate a short distance as neuroblasts and thus become integrated as granule neurons in the granular cell layer of the dentate gyrus (Cameron *et al.*, 1993; Eriksson *et al.*, 1998; Kempermann *et al.*, 2004a). Studies confirming the existence of hippocampal neurogenesis in the non-human primates such as in the adult macaques, evidence for continuous generation of neurons, oligodendrocytes and astrocytes in the dentate gyrus was reported by Kornack and Rakic (1999). The authors found that the immature bipolar shaped cells observed in the subgranular zone layer resembled that of migrating young neurons during the hippocampal development in young macaque monkeys.

Several studies provided evidence of adult neurogenesis in the dentate gyrus and established that it is a feature of all mammalian species. This has been demonstrated in humans (Erikson *et al.,* 1998); in treeshrews (Gould *et al.,* 1997); in Marmosets (New World Monkeys) (Gould *et al.,* 1998; Sawamoto *et al.,* (2011) and Marlatt *et al.,* 2011) and in Macaques (Old World Monkeys) (Gould *et al.,* 1999a). Kornack and Rakic (1999) also reported that the rate of adult hippocampal neurogenesis was found to be 10 times higher in adult rodents than in adult macaques.

### 2.2.2. POTENTIAL NEUROGENIC SITES

**Cerebellum**

Adult neurogenesis in the cerebellum of non-mammalian vertebrates is reported to occur throughout life (Kaslin *et al.,* 2008). In contrast to this the mammalian cerebellar neurogenesis was reported to become static after early postnatal genesis of granule cells and was believed to be limited to the early post-natal period (Das and Nornes, 1972). The genesis of most cerebellar cell types is reported to occur from the periventricular neuroepithelium lining the $4^{th}$ ventricle (Bonfanti and Peretto, 2011). Altman (1969) established that in rodent's cerebellar cortex, the first cells to differentiate were basket cells, and were situated in the lower half of the molecular layer followed by differentiation of the stellate cells and lastly the granule cells. The delayed genesis of granule cells is considered as a critical period that allows formation of new synaptic connections as well as the addition of new neurons (Bonfanti and Peretto, 2011).

A study by Das and Nornes (1972) reported that granule, basket and stellate cells were primarily in the nodulus flocculus and paraflocculus. The external granule cell layer (EGL) is formed by displacement of cell precursors from the germinal trigone of the $4^{th}$ ventricle (Bonfanti and Peretto, 2011). In rabbit the cell proliferation is reported to extend beyond

puberty, and the germinal layer of which such proliferation occur, was named the subpial layer (Ponti *et al*., 2006) and has features that are different from the external granule cell layer (EGL) (Bonfanti and Peretto, 2011). This layer (subpial) is characterized by occurrence of tangential chains of neuroblasts which were reported to share the same cytology as those observed in SVZ of the forebrain in mice and rabbits (Ponti *et al.*, 2006; 2008). No cell proliferation was reported in the ependymal layer of the fourth ventricle in both rabbits and rodents postnatally (Ponti *et al.*, 2010). Cerebellar neurogenesis was also reported in species like the adult Goldfish (Delgado and Schmacternberg, 2011); New Zealand white rabbit (Ponti *et al.*, 2006); Zebrafish (Kaslin *et al.*, 2008) . So far no study was able to detect any neurogenesis in the human cerebellum (Bhardwaj *et al.*, 2006; Bergmann *et al.*, 2012).

**Cerebral cortex**

Adult neurogenesis exists in various areas of the cerebral cortex in non-human primates. For several decades mammalian studies reported newly generated neurons in the neocortex (Altman, 1963; Huang *et al.*, 1998; Gould *et al.*, 1999a,b; Gould *et al.*, 2001; Bernier *et al.*, 2002; Fowler *et al.*, 2002; Dayer *et al.*, 2005; Yang *et al.*, 2015). Gould and colleagues (1999a, b) reported that streams of new neurons were generated and added to the association areas of the cerebral neocortex in the adult macaque monkeys *(Macaca fascicularis)*. Consecutively, Gould and colleagues (2001) conducted another study and observed adult-generated cells in the white matter of the macaque monkeys and these cells were reported to be immature neurons that were migrating from the SVZ to the cerebral cortex. Also adult neurogenesis was reported in other regions of the cortex such as the prefontal cortex, inferior temporal cortex and parietal cortex but not reported in the striate cortex (Gould *et al.*, 1999a; b and Gould *et al.*, 2001).

In contrast to the above findings, Kornack and Rakic (2001) conducted a similar study using BrdU in adult macaque monkeys, and found that BrdU labeled cells were non neuronal, and

they did not detect newly generated neurons in the neocortex nor the streams of migratory neurons from the SVZ to the prefrontal cortex. They further reported that there is no neurogenesis in the white matter of macaque monkeys and that adult neurogenesis was limited to the hippocampus and the olfactory bulb.

In agreement with the above findings, Koketsu and colleagues (2003) conducted a study in the juvenile and adult Macaque monkeys, and did not detect BrdU positive neurons in the cerebral neocortex nor migratory neurons or addition of new neurons in the frontal cortex. However the detected cells were said not to be newly generated neurons but were of glial and endothelial nature, thus they concluded that in the cerebral cortex of healthy mature primates neurogenesis was stable and neurons were neither supplementary nor non - renewable.

Other most recent studies were conducted through the use of $^{14}C$ birth dating in human primates, and lack of neurogenesis in the neocortex was reported (Spalding *et al.*, 2005; Bhardwaj *et al.*, 2006). However the authors reported this was found to be restricted to developmental stage. Huttner and colleagues (2014) also reported that no adult neurogenesis was observed in various areas of the cortex of human subjects who suffered ischemic stroke. Yang and colleagues (2015) also found the DCX expressing cells in the cerebral cortex of the guinea pigs and discovered that these cells orderly resided in deep to superficial layers of the neocortex.

**Piriform cortex and Amygdala**

In mammals the piriform cortex receives input from the olfactory bulb and reciprocally sends efferent fibers through the entorhinal cortex to the hippocampus (Klempin *et al.*, 2011). More studies reported cells in the second layer of the piriform cortex and that this were generated during the adult phase (Bernier *et al.*, 2002; Pekcec *et al.*, 2006; Shapiro *et al.*, 2007; Kemplin *et al.*, 2011).Bernier and colleagues (2002) conducted a study in the adult

cynomolgus (*Macaca fascicularis*) monkeys and reported evidence of newly generated neurons surrounding the piriform cortex. . Other studies like that of Nacher and colleagues (2002) in adult rats, Luzzati and colleagues (2009) in rabbits did not report evidence of new neurons being incorporated in the piriform cortex.

Few primate studies confirmed adult neurogenesis in the amygdala. Studies by Bernier and Parent (1998 a, b) reported evidence of adult neurogenesis in the amygdala of the adult squirrel *(Saimiri sciureus)* monkeys. Another study by Bernier and colleagues (2002) reported evidence of new neurons that were thought to migrate from the temporal lobe SVZ to the amygdala of the adult cynomolgus *(Macaca fascicularis)* monkeys

**Striatum**

Many mammalian studies ranging from rodents ( Dayer *et al.*, 2005) to primates (Bedard et al., (2002a, b; 2006). Bedard et al., (2002a, b) and Berdad *et al.*, (2006) showed the existence of neurogenesis in the striatum of adult squirrel monkeys; however the authors established that the number of newly generated striatal neurons was very small under normal conditions. Dayer and colleagues (2005) reported neurogenesis in the striatum of rodents.

Despite this, when they used brain derived neurotropic factor (BDNF) treated with BrdU, numerous BrdU positive cells were found within the striatum. The authors also found numerous BrdU positive cells located near the SVZ, and these cells co-expressed polysialylated neural cell adhesion molecule (PSA-NCAM) and DCX. Thus they theorized that this discovery suggests that precursor cells migrate from the lateral ventricle to the striatal parenchyma and develop a neuronal phenotype which is similar to that in rodents (Berdard *et al*., 2006; Bonfanti and Peretto, 2011). Also these cells were reported to be mostly found in the nucleus accumbens, the dorso-medial striatum, and the caudate nucleus in non- rodent mammals (Bonfanti and Peretto, 2011).

Ernst and colleagues (2014) through the use of 14 carbon dating were able to established that adult neurogenesis in humans not only occurs in the lateral ventricle wall but also in the striatum and that the neuronal turnover was about 2.7% and appeared to be restricted to interneurons. The authors also established that in Huntington's disease patients, postnatally generated neurons were reported to be absent in the advanced stages of the disease.

### 2.3. Justification of the study

Most studies of adult neurogenesis have been conducted in rodents, less in non-human primates and few in humans. In most rodents investigated, cell proliferation was reported to be fairly high. At present, the rodent findings are generally extrapolated to the situation in humans; however the few primate and human studies have shown that adult neurogenesis occurs at much lower levels than seen in rodents (Balu and Lucki, 2009).The contrasting results of the studies from different laboratories sparked the need for similar studies to be undertaken in other laboratories and more primates studies needed to be undertaken. Since it was assumed that the negative staining might be related to the type of immunohistochemical marker, more less toxic immunohistochemical markers other than BrdU needed to be investigated. It was further suggested that the contrasting results from other laboratories might be due to the capture stress or handling of an animal or other physiological stimuli. In this study the effects of capture stress was minimized by euthanizing the animals within 8 hours of being captured because the longer the animal stays in a stressful situation the lesser the rate of neurogenesis. The animals used for this study were from different environmental niches (wild and zoo kept).

No previous studies of adult neurogenesis have been undertaken in any prosimian species. The three species of Prosimians targeted for investigation in the current study are Potto and Galago which are nocturnal and the Lemurs (*Lemur catta*) which are diurnal. Phylogenetic studies placed Prosimians within the primate order like humans; therefore they are expected to share some common neural characteristics with humans.

### 2.4. Main Aim

To investigate and describe the presence and pattern of adult neurogenesis in the brains of three prosimian primate species, namely the Galago (*Galagoides demidoff phasma*); Potto (*Perodictus potto*) and the Lemur (*Lemur catta*).

### 2.5. Specific Objectives

1. To establish whether adult neurogenesis occurs in active neurogenic sites such as the SVZ of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus of Prosimian primates.
2. To determine whether immature or maturing neurons are found in potential neurogenic sites.
3. To characterize the morphology of the newly generated neurons as revealed by the DCX immunohistochemistry.
4. To estimate the rate of cell proliferation in the dentate gyrus of the hippocampus through the counting of Ki-67 positive cells

## CHAPTER 3

## MATERIALS AND METHODS

### 3.1. The experimental animals

Primates emerged as a distinct line of evolution over several million years ago and formed the three major evolution radiations, namely the prosimians, the tarsoids and the anthropoids (Wu & Kaas, 2003 and Kaas, 2009) (See figure 3.1 below). The anthropoid radiation led to New world and Old world monkeys, and Apes including humans. The prosimian radiation led to Lemurs and Lorises (Figure 3.1). The prosimians where further classified into a family of Lemuridae, Galagidae and Lorisidae (Table 3.1).

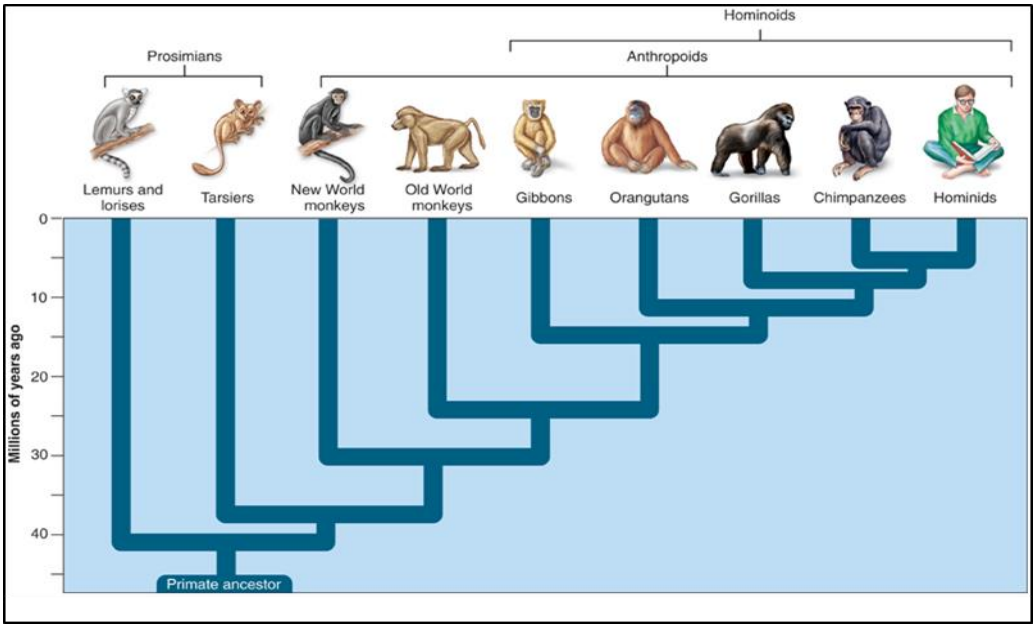


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**Figure 3.1**: A family tree showing the evolution of the primates from a common primate ancestor.

**Table 3.1** Taxonomic classification of the Prosimian primates

| Order | Suborder | Infraorder | Superfamily | Family | Genus | Scientific name |
|---|---|---|---|---|---|---|
| Primates | Strepsirhinni | Lemuriformes | Lemuroidea | Lemuridae | Lemur | *Lemur catta* |
| | | Lorisiformes | Lorisoidea | Galagidae | Galago | *Galagoides demidoff phasma* |
| | | | | Lorisidae | Perodictus | *Perodictus potto* |

### 3.1.1 The Ring Tailed Lemur *(Lemur catta)*

The Ring tailed lemurs are the largest strepsirrhine primates and the only members of the genus Lemur (see Table 3.1). They are diurnal animals who spend most of their time on the ground or open spaces. Their habitat is mostly terrestrial, closed canopy forest, dry bush and scrubs. The Lemurs are the most endangered species due to loss of habitat, degradation, hunting and endemism because they are only found in the south central and southwest parts of the Madagascar Island (Garbutt 1999; Thernstrom, 2010). However housing of animals in the zoo has played an important role in improving the welfare of endangered species. More than 300 zoos all over the world are housing the Lemurs (Thernstrom, 2010).



**Figure 3.2**: Ring tailed Lemur (Lemur Catta). Photographed in captivity at Faunia (Madrid) by Gloria Fernadez Lazaro. Adopted from Gloria Lazaro (US-EU) project on Animal Welfare Science, Research Institute of North American Studies, University of Alcala de Henares.

The average size of Ring tailed lemur is shown the Table below (Nowak, 1991).

**Table 3.2**: Table showing the general estimations on morphometric measurements of the adult Lemur

| **Body Parameters** | **Values** |
|---|---|
| Head and body length | 385mm – 455 mm |
| Tail length | 560 mm – 624 mm |
| Body weight | 1.15 kg – 2.45 kg |

Lemurs are omnivorous animals whereby their diet consists of fruit, leaves flowers and bark, spiders, birds and caterpillars. They are macrosmatic animals that display a complex array of scent marking behaviour (Drea and Scordato, 2008). Both males and females have apocrine and sebaceous glands in their genital areas with males having additional specialized glands

on their wrists and pectoral surfaces (Garbutt, 1999; Drea and Scordato, 2008; Thernstrom 2010) They heavily rely on olfactory communication in such way that they perform odour cues to mark territories, advertise resources ownership, signal reproductive state and maintain intersexual dominance. (Garbutt,1999; Thernstrom 2010). Lemurs live together in multifemale and multimale social structures, however females are found to be more dominant than males regardless of rank (Drea, 2007). Females often advertise their readiness to mate by showing off external genitalia (Drea and Scordato, 2008). Males reach sexual maturity at about 3 to 4 years of age (Thernstrom, 2010).

### 3.1.2 Potto (*Perodictus Potto*)

Potto is the largest member of the Loridae family. They are nocturnal, arboreal animals whose habitat ranges from the lowlands, swamp, and riverine to montane forests. They are found across the tropical forest belt of western, central and eastern Africa (Nowak, 1991; Burton and Burton, 2002; Butynski and de Jong, 2007).The average size of an adult Potto is shown on the Table 3.3 below (Nowak, 1991).

**Table 3.3**: The general estimation on Morphometric measurements of adult Potto Body Parameters

| **Body Parameters** | **Values** |
|---|---|
| Head and body length | 305 mm – 390 mm |
| Tail length | 37 mm – 100 mm |
| Body weight | 850 g – 1600 g |



**Figure 3. 3** : A photomicrograph of the Perodictus Potto taken by Professor Paul Manger (April 2012).

The diet of Potto consists of mainly fruits, nectar, gums, snails, insects, eggs and fungi. Like the Galago communication is also mostly dependent on olfactory communication. The other means of communication between mother and young is through high pitched 'tsic' vocalizations. Whistling calls are often used by females during estrus periods and groaning in threatening situations (Nowak, 1991; Burton and Burton, 2002; Macdonalds, 2006; Butynski and de Jong, 2007).

The female oestrus cycle averages 39 days with the gestation period of about 180 – 205 days. They usually give birth to a single infant with birth weight ranging from 30 – 52 grams (Nowak, 1991). The young is carried on the female's belly or left hidden on a tree branch until it is weaned at about 4 to 5 months. The young reaches maturity between 9 to 18 months

and may live up to 11 years in the wild and 26 years in captivity (Nowak, 1991; Butynski and de Jong, 2007).

### 3.1. 3 Galago (*Galagoides demidoff phasma*)

Dwarf galagos are one of the smallest primates within the Galagidae family (Nowak, 1991). They are arboreal and nocturnal animals who spend the rest of the day sleeping in fully enclosed leaf nests which are about 5 to 40 metres above the ground and are usually found from Senegal across to western Tanzania, in the Democratic republic of Congo and on Bioko Island in the Gulf of Guinea (Nowak, 1999; Butynski and de Jong, 2007).



**Figure 3.4**: Photomicrograph of the Galago. Picture by Bruce Davidson. www.arkive.org accessed 06/04/2012.

When hunting for food, Galagos use their acute senses to follow inconspicuous prey through the dense foliage. Their diet consists of fruits, gums, leaves, buds and small insects such as beetles, caterpillars and moths (Nowak, 1991). The males are aggressive and dominant, heavier males control home ranges that have more females. The females have one pregnancy each year, with the gestational period of about 133 days. The infant weight ranges from 5 to 10 grams at birth and is usually weaned at around 6 weeks and reaches sexual maturity

between 8 and 11 months (Nowak, 1991). The average size of an adult Galago is indicated on the table 3.4.

**Table 3.4**: The general estimation on Morphometric measurements of the adult Galago.

| Body Parameters | Values |
|---|---|
| Head and body length | 73 mm – 155 mm |
| Tail length | 110 mm – 215 mm |
| Body weight | 44 g – 97 g |

Communication in the Galago is mostly by olfactory communication than sight which is achieved through the scent marking behaviour such as urine washing and glandular secretions (Nowak, 1991; Butynski and de Jong, 2007). Females are believed to use the scent marking behaviour during their oestrus period to advertise their readiness to mate and for attraction of sexual partners. Males often use the behaviour for territorial marking.

## 3.2 Experimental procedure

### 3.2.1 Tissue processing

The Galago and the Potto used for this study were captured from wild populations in the Yoko rainforest, south of Kisangani in the Democratic Republic of Congo. The permissions to capture these animals were obtained from the Kenya National Museums and the Kenyan Wildlife Services, and the University of Kisangani. The Lemurs were captured from the breeding colony of the Copenhagen zoo and permission was obtained from the Copenhagen zoo.

All animals were treated and used in accordance with the University of the Witwatersrand Animal Ethics and Screening Committee guidelines (clearance number: 2008/36/1). The Galago and Potto were captured at night and within 8 hours of capture were euthanized and perfused through the left ventricle with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The Lemur was captured from the zoo during the day and was euthanized and perfused immediately it was captured. Two animals from each species were used in this study.

The brain was removed, weighed and post-fixed in 4% paraformaldehyde overnight, cryoprotected in 30% sucrose in 0.1 M PB at 4 $^{o}$C and stored in an antifreeze solution at - 20 $^{o}$C until sectioning. Two brains from each species were used, whereby one brain was used whole for coronal sections and the other was cut in the midsagittal plane for sagittal sections. For the morphometric values of the experimental see Table 3.5 below.

**Table 3.5**: Morphometric values of Experimental animals.

| Species | Body weight | Brain mass | Section |
|---|---|---|---|
| Lemur | 1.75 kg | 21.50 g | Sagittal |
| | Unknown | 24.11 g | Coronal |
| Potto | 1.4 kg | 13.94 g | Sagittal |
| | 1.4 kg | 12.79 g | Coronal |
| Galago | 74 g | 2.85 g | Sagittal |
| | Unknown | 3.27 g | Coronal |

Before sectioning, the tissue was allowed to equilibrate in 30% sucrose in 0.1 M PB at 4 $^{o}$C for a period of about 48 hours. The specimen was frozen in crushed dry ice and sectioned into 50 µm thick sections on a sliding microtome. For coronal sections a needle was used to mark

the hemisphere in order to indicate the side and for proper orientation before freezing the specimen in the dry ice. For this study the right hemisphere was marked. For sagittal sections the left hemisphere was used and a one-in-three series of sections was used for immunostaining in all three species. For coronal sections, a one-in-eight series was used for immunostaining in the Galago species whereas in Potto and the Lemur a one-in-ten series was used for immunostaining.

### 3.2.2 Cresyl Violet staining

The first section of each series was mounted on 0.5% gelatine-coated slides, dried overnight, cleared in a 1:1 mixture of 100% ethanol and 100% chloroform overnight, and then rehydrated in alcohol by placing the slides in a series of alcohol baths of decreasing concentration. The slides were placed in 100% for 5 minutes, then another 100% for 5 minutes, 95% for 2 minutes, 70% for 2 minutes and 50% for 2 minutes. The slides were then placed in 1% Cresyl Violet for 2 minutes before being transferred into distilled water for 2 minutes. Sections were then dehydrated in graded alcohol baths of increasing concentration, for the same time periods as recorded above (i.e. starting in 50% alcohol up to 100% alcohol).

The only exception was the 70% alcohol bath, this step acts as a differentiation step, therefore the amount of time in which the slides remained in the bath was dependent on the intensity of cresyl violet stain. The staining was monitored under stereomicroscope until optimal staining was achieved, and then dehydration continued. After the last dehydration step (5minutes in 100% alcohol) the slides were cleared in xylene and cover slipped with DPX mountant (Merck (Pty) Ltd, Wadeville, Gauteng, South Africa).

### 3.2.3 Immunohistochemistry for Doublecortin (DCX)

Doublecortin is a microtubule protein that is usually found in migrating neuroblasts and young neurons and it is responsible for promoting microtubule polymerization (von Bohlen et al., 2007). During the initial stages of neuronal differentiation in the hippocampus, DCX is expressed and the cells are reported to be unpolarised and their processes are usually found parallel to the granule cell layer, however towards the end of cell differentiation the cells become highly polarized with processes projecting into the molecular layer (Plumpe et al, 2006).

The sections were incubated in a 1.6% $H_2O_2$, 49.2% methanol, 49.2% 0.1 M PB solution, for 30 minutes to reduce endogenous peroxidase activity, which was followed by three times 10-min rinses in 0.1 M PB. To block unspecific binding sites the sections were then pre-incubated for 2 hours, at room temperature, in blocking buffer (3% normal rabbit serum – NRS, plus 2% bovine serum albumin - BSA, and 0.25% Triton X-100 in 0.1 M PB) Thereafter, the sections were incubated for 48 h at 4 $^{o}$ C in the primary antibody solution (1:300, goat anti-doublecortin, DCX, SC-18 Santa Cruz Biotech, Santa Cruz, California, USA) under gentle shaking.

The primary antibody incubation was followed by three times 10-min rinses in 0.1 M PB. The sections were then incubated in a secondary antibody solution (anti-goat IgG, BA 5000 for DCX, Vector Labs, Burlingame, California, USA, in a mixture of 3% NRS and 2% BSA in 0.1 M PB) for 2 hours at room temperature. This was followed by three times 10-min rinses in 0.1 M PB, after which sections were incubated for 1 hour in an avidin-biotin solution (AB complex) (1:125; Vector Labs), followed by three times 10-min rinses in 0.1 M PB. The sections were then incubated in a solution containing 0.05% diaminobenzidine (DAB) in 0.1 M PB for 5 min, followed by the addition of 3.3 µl of 30% hydrogen peroxide

per 1 ml of DAB solution. Chromatic precipitation was visually monitored under a low-power stereomicroscope until the background stain was at a level that would allow for accurate architectonic matching to the Nissl sections without obscuring the immunopositive structures. Development was stopped by incubating sections in 0.1 M PB for 10 min, followed by two more 10-min rinses in this solution. Sections were then mounted on 0.5% gelatin-coated glass slides and were allowed to dry for a period of 48 hours. The dried sections were then dehydrated in a graded series of alcohols, 70 % for 2 hours; 95 %for 2 minutes; 100 % for 5 minutes; 100 % for 5 minutes. Then cleared in Xylene for 5 minutes, and cover slipped with DPX mountant and were allowed to dry before the analysis.

### 3.2.4 Categorization of DCX positive cells

To characterize the morphology of the DCX positive cells, the method from a study by Plumpe et al (2006) was adopted. The DCX positive cells were classified into six categories according to the shape and presence of apical dendrites. The categories were as depicted in Table 3.6.

**Table 3.6**: Summary of categorization of DCX cells

| Category | Neuron type |
|---|---|
| A | Immature cells with no processes |
| B | Immature cell with very short processes (length less than one nucleus wide) |
| C | Immature cell with processes equal to one nucleus wide and not touching the molecular layer |
| D | Immature cell with intermediate process which is longer than one nucleus wide and touching the molecular layer |
| E | Cell showing mature appearance with one dendrite penetrating the molecular layer and showing sparse dendritic branching |
| F | Cell showing mature appearance with dendrite reaching the molecular layer and showing dense branching with few branches near the soma or within the granule cell layer. |

### 3.2.5 Immunohistochemistry for Ki-67

Ki-67 is an endogenous marker that is expressed at different levels during mitosis. It is reported to be expressed during mitosis in all mammalian species, from rodents to humans (Amrein et al, 2002). A study by Amrein et al (2002) compared BrdU and Ki-67 labelling and established that the Ki-67 method detected 50 % more neurogenic cells than BrdU. Moreover this was an expected outcome since Ki-67 is expressed in all stages of mitosis except the early G1 phase. Also compared to BrdU it was established that Ki-67 does not have any adverse effects on living cells, therefore Ki-67 immunostaining was used to estimate the rate of proliferative cells in the various parts of the brain.

The sections were incubated in a 1.6% $H_2O_2$, 49.2% methanol, 49.2% 0.1 M PB solution, for 30 minutes to reduce endogenous peroxidase activity, which was followed by three times 10-min rinses in 0.1 M PB. To block unspecific binding sites the sections were then pre-incubated for 2 hours, at room temperature, in blocking buffer (3% normal goat serum – NGS, plus 2% bovine serum albumin – BSA, and 0.25% Triton X-100 in 0.1 M PB). Thereafter, the sections were incubated for 48 h at $4^{o}$ C in the primary antibody solution (1:1000, rabbit anti-Ki-67, NCL-Ki-67p DAKO, Glostrup, Denmark) under gentle shaking. The primary antibody incubation was followed by three times 10-min rinses in 0.1 M PB. The sections were then incubated in a secondary antibody solution (1:1000 dilution of biotinylated anti-rabbit IgG, BA 1000, in a mixture of 3% NGS and 2% BSA in 0.1 M PB) for 2 hours at room temperature. This was followed by three times 10-min rinses in 0.1 M PB, after which sections were incubated for 1 hour in an avidin–biotin solution (AB complex)

(1:125; Vector Labs), followed by three times 10-min rinses in 0.1 M PB. The sections were then incubated in a solution containing 0.05% diaminobenzidine (DAB) in 0.1 M PB for 5 min, followed by the addition of 3.3 µl of 30% hydrogen peroxide per 1 ml of DAB solution. Chromatic precipitation was visually monitored under a low-power stereomicroscope until the background stain was at a level that would allow for accurate architectonic matching to the Nissl sections without obscuring the immunopositive structures. Development was stopped by incubating sections in 0.1 M PB for 10 min, followed by two more 10-min rinses in this solution. Sections were then mounted on 0.5% gelatin-coated glass slides and were allowed to dry for a period of 48 hours. The dried sections were then dehydrated in a graded series of alcohols, 70 % for 2 hours; 95 %for 2 minutes; 100 % for 5 minutes; 100 % for 5 minutes. Then cleared in Xylene for 5 minutes, and cover slipped with DPX and were allowed to dry before the analysis.

### 3.2.6 Proliferating cell count

In all species studied, a cell count of proliferating cells was performed on the left hippocampus in all sections. For quantitative analysis, the rate of AHN cell proliferation was estimated through the physical counting of Ki-67 positive cells in the dentate gyrus. All Ki-67 positive cells in every fourth section were quantified and only cells in the subgranular layer and granule cell layer of the selected left hemisphere were counted. The method was adapted from Ajao et al, (2010), whereby proliferating cells that can be identified were counted using the Axiovision Light microscope with a 63 X objective and multiplied by the section sampling fraction to obtain the estimated total proliferating cell number.

### 3.2.7 Data analysis

The staining patterns of Ki-67 and DCX were observed using low-power stereomicroscope (0.63 X objective lens) (Leica Mz75, Leica microsystems Ltd, CH, 9435, Heerbugg, Switzerland) and architectonic borders were traced according to the Nissl-stained sections using a camera lucida. One in four sagittal sections was selected for drawing. The Ki-67 and DCX staining patterns were then matched to the drawing from the traced Nissl-stained sections. Selected drawings were then scanned and redrawn using the Canvas 7 Software (Deneba Software, Miami, Florida, USA). Digital photomicrographs were captured using Zeiss Axioshop (Carl Zeiss Microscopy GmbH, Jena, Germany) and Axiovision software (Carl Zeiss Microscopy GmbH, Jena, Germany). For proper representation of results one in four sagittal sections not selected for drawing was selected for photomicrographs. In the coronal sections one in four was selected for photomicrographs.

The distribution of the data was explored and established that it was normally distributed, then a student t-test was performed in order to determine if the means in the rate of proliferative cells was statistically different within the three species. In each species the following number of sections was obtained for statistical analysis: in Potto 20 coronal sections and 23 sagittal sections, Galago 12 sagittal sections and 14 coronal sections and in the Lemur 23 coronal sections and 26 sagittal sections.

# CHAPTER 4: RESULTS

The results in the current study confirmed that adult neurogenesis occurs in the established sites and potential sites in all the three prosimian species studied.

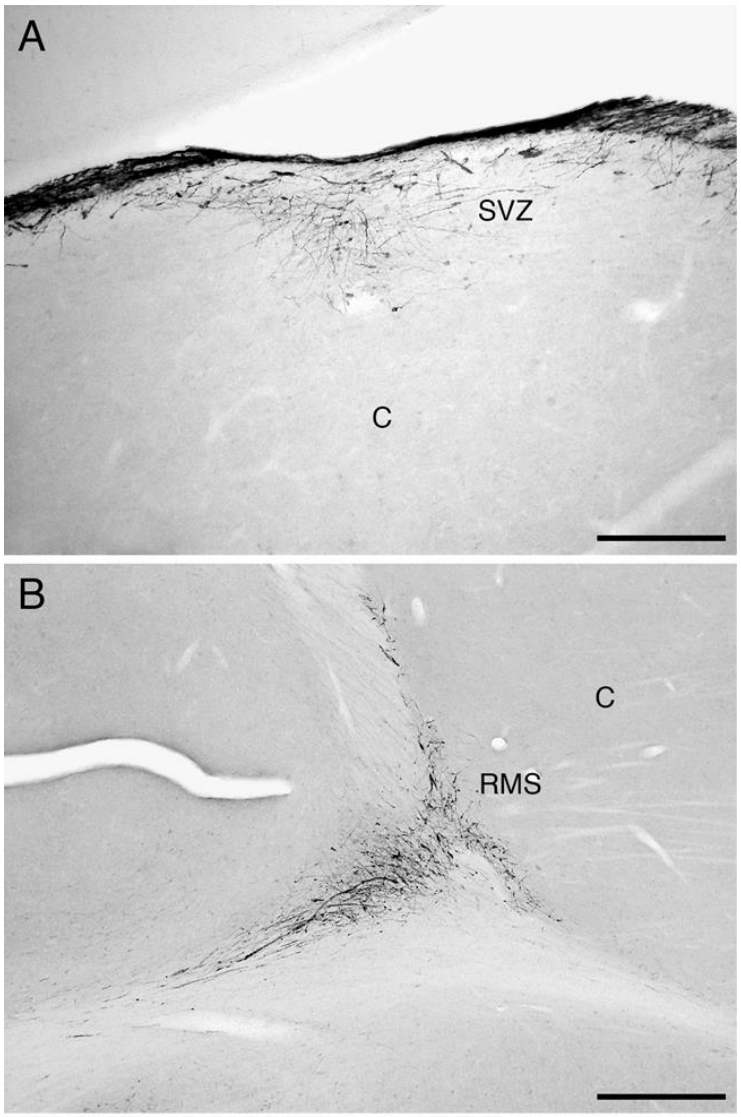
## 4.1 DCX positive cells in established neurogenic sites

The established neurogenic sites are the sub-ventricular zone of the lateral ventricle and the dentate gyrus of the hippocampus.

**The Subventricular zone (SVZ) of the lateral ventricle, the rostral migratory stream (RMS) and the olfactory bulb (OB).**

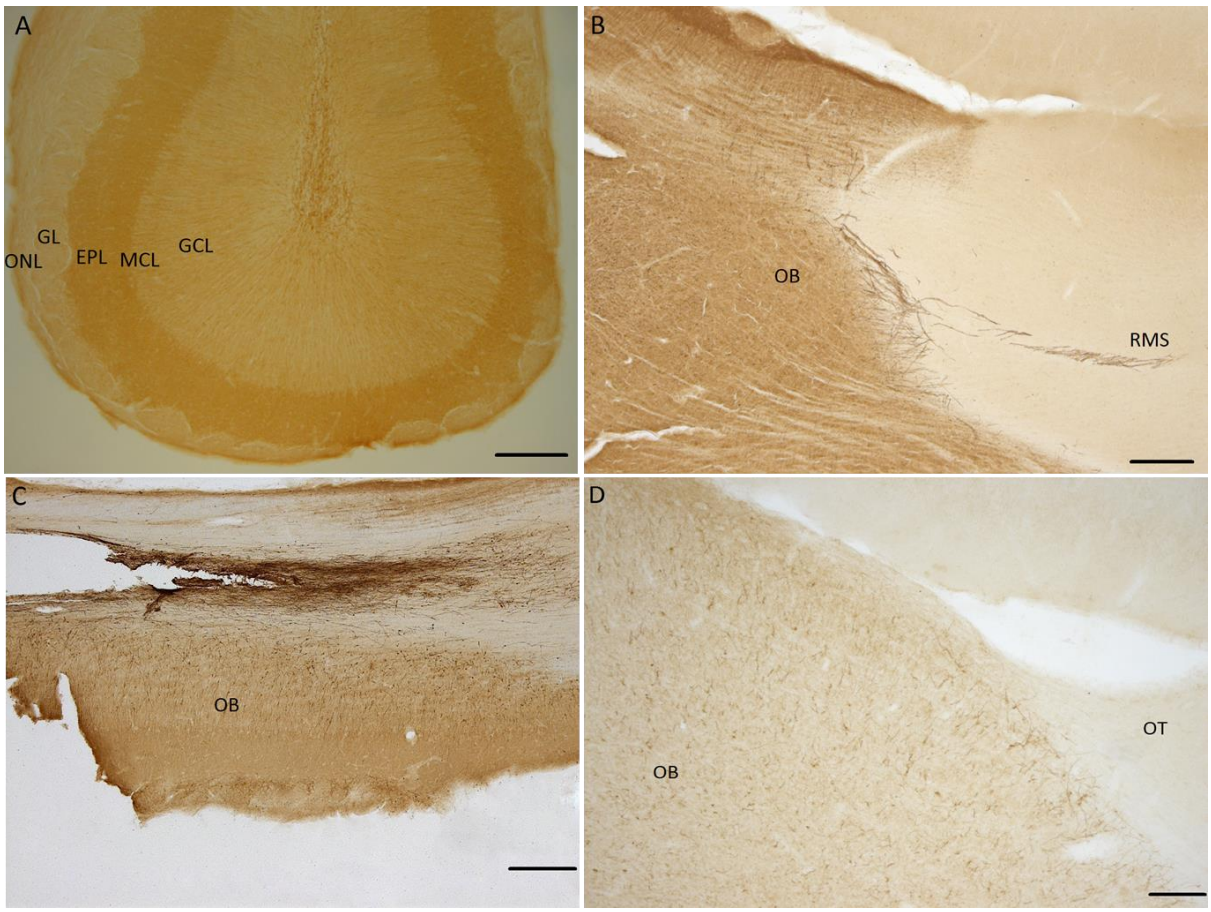
DCX positive cells were observed in the SVZ of the lateral ventricle (Figure 4.1 A) in all the three species, and were observed moving in migratory chains along the rostral migratory stream (RMS) (Figure 4.1B) towards the Olfactory bulb (OB). The descending limb of the RMS was coursing ventro-caudally from the SVZ along the anterior boundary of the caudate nucleus to the sub-cortical white matter of the anterior olfactory cortex and the rostral limb extended ventro-rostrally into the olfactory tract to the olfactory bulb (OB) (Figure 4.1B).

**Figure 4.1**: Representative Photomicrographs of the positive DCX cells in different areas of the brain of the Lemur. A = positive cells in the sub ventricular zone of the lateral ventricle and B= showing positive cells moving along Rostral migratory stream (RMS). Scale bar: A and B = 250μm


A
SVZ
C
B
C
RMS

The prosimian primate olfactory bulb reduced in size as the brain of the animal increased, it consisted of six layers that could be differentiated microscopically. The outer most layer consisted of thin olfactory epithelium, followed by the olfactory nerve layer (ONL). The glomerular layer (GL) was found deep into the ONL and followed by the External plexiform layer (EPL).The mitral cell layer (MCL) was in between the EPL and the granule cell layer (GCL). DCX positive neurons were observed in the olfactory bulb with more interneurons observed in the GCL and migrating radially to outer layers. In all the species the cells were densely concentrated at the olfactory granular cell layer and became distributed radially to the outer layers of the olfactory bulb (Figure 2.A). The results as depicted in the Figure 4.2 showed that positive DCX cells were observed in the olfactory bulb of the Galago (Figure 2.A & B), the Lemur (figure 4.2 C ) and the Potto (Figure 4.2 D).

**Figure 4.2**: Photomicrographs showing the DCX positive cells in: A the Olfactory bulb (OB) of the Galago, B = the Rostral migratory stream (RMS) of the Galago, C = the olfactory bulb (OB) of the Lemur and D= cells in the OB of the Lemur and D = cells in the OB of Potto. OT = olfactory tubercle. Scale bar= 500µm and D = 250µm.

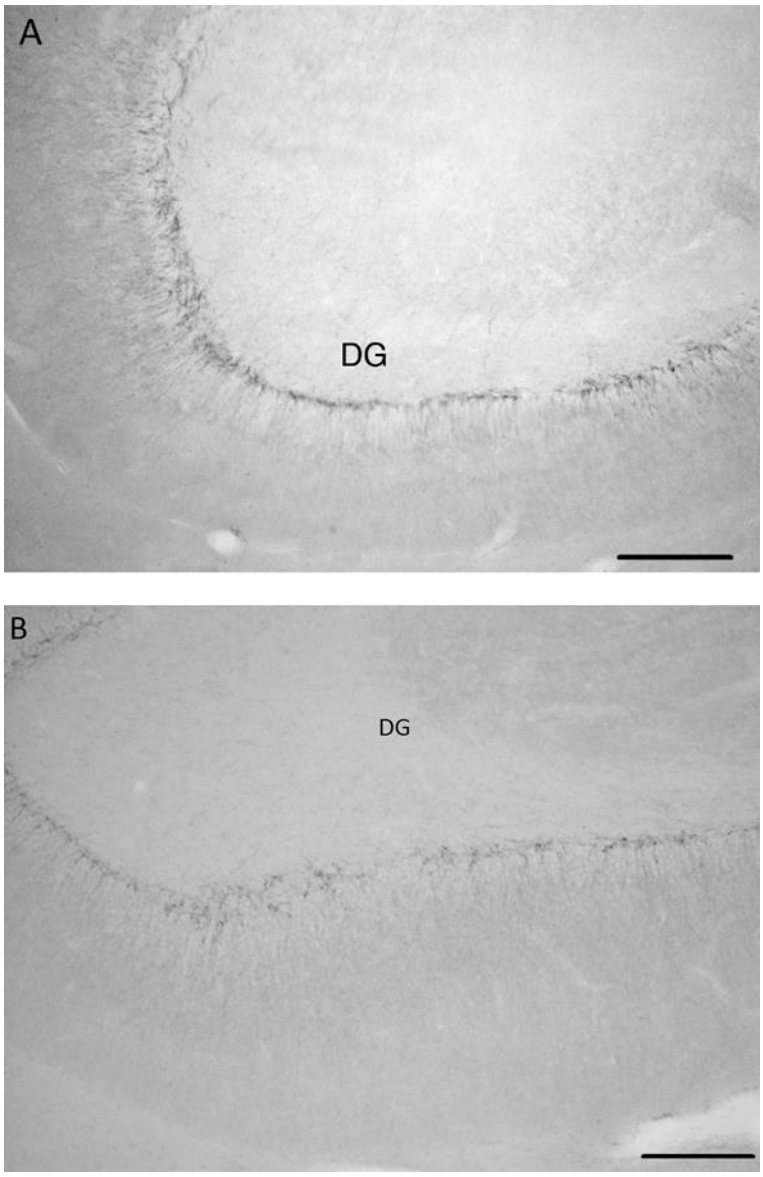

A
GL
ONL
EPL
MCL
GCL
B
OB
RMS
C
OB
D
OT
OB

**The dentate gyrus of the hippocampus**

Positive DCX cells were observed in the dentate gyrus (DG) of all the investigated species. Most cells were found with their cell bodies embedded in the subgranular zone. These positive cells were migrating in the granular cell layer of the dentate gyrus (DG), and differentiating into granule neurons with some of the neurons having their dendrites penetrating the molecular layer. In the Lemur, most of the positive cells had densely branched dendrites and were observed to be penetrating the molecular layer. More so the projections of these granule neurons were extending more to the CA3 region of the hippocampus in the Lemur compared to the Potto and the Galago.

Some similarities observed in the DCX positive cells of the Potto and the Galago, most of their cells had their cell bodies embedded in the sub-granular zone layer and had the single axon extending to the granule cell layer (Figure 4.3A & B). The density of DCX positive cells was more in the Potto compared to the Galago. Also more multipolar cells were visible in Potto with few in the Galago.

**Figure 4.3**: Photomicrographs showing the DCX positive cells in the dentate gyrus of the Galago (A) and Potto (B). Scale bar= 250 µm.

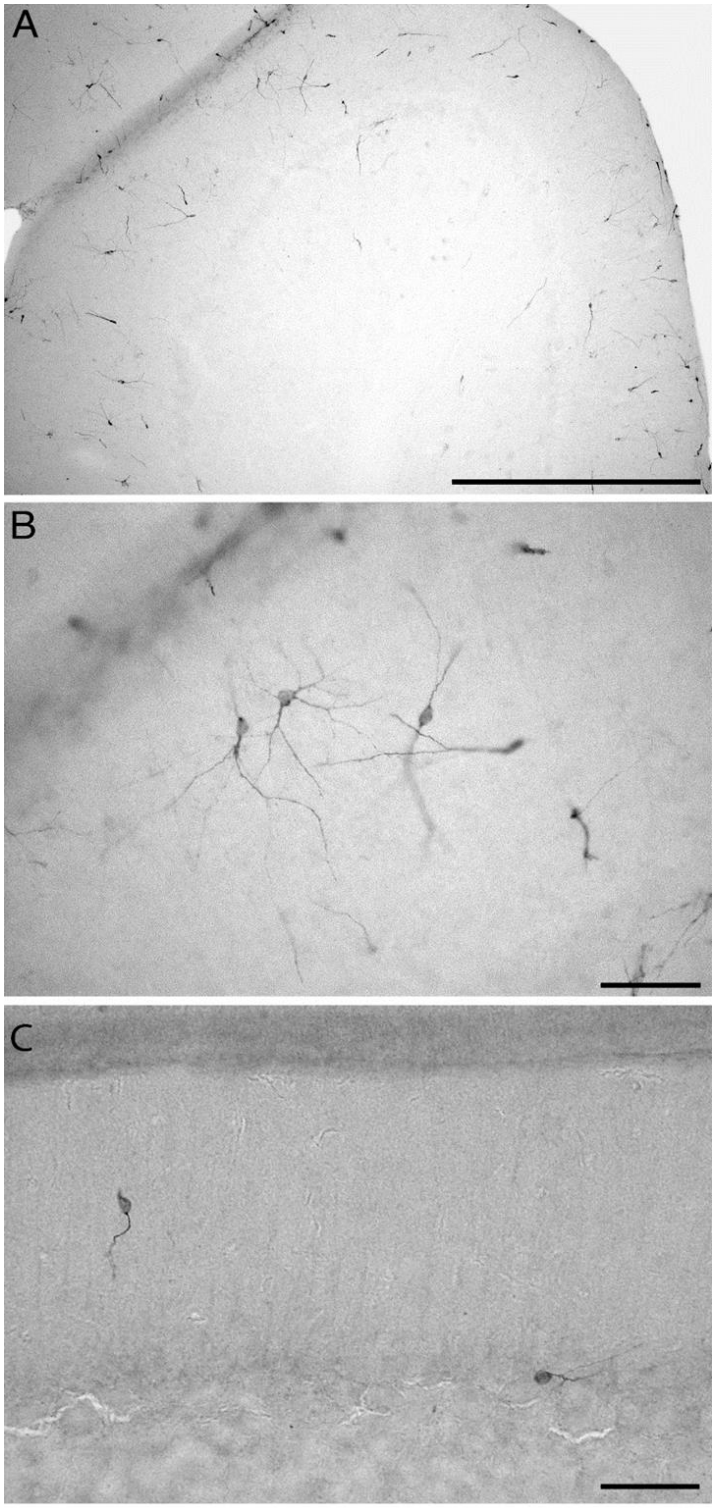

A
DG
B
DG

## 4.2 DCX positive cells in the potential sites

**The Cerebellum**

The DCX positive cells were observed in the cerebellum of the Lemur and Galago while in the Potto none was observed. In the Lemur the cells were mostly of mature appearance, with their densely branched dendrites on the cerebellar cortex (Figure 4.4A). Some of these cells were multipolarized with their dendrites penetrating the molecular layer of the cerebellum (Figure 4.4B). However in the cerebellum of the Galago (Figure 4.3C), DCX positive cells were more of immature appearance with a single process and no dendritic branching. No positive DCX neurons were observed in the cerebellum of Potto.

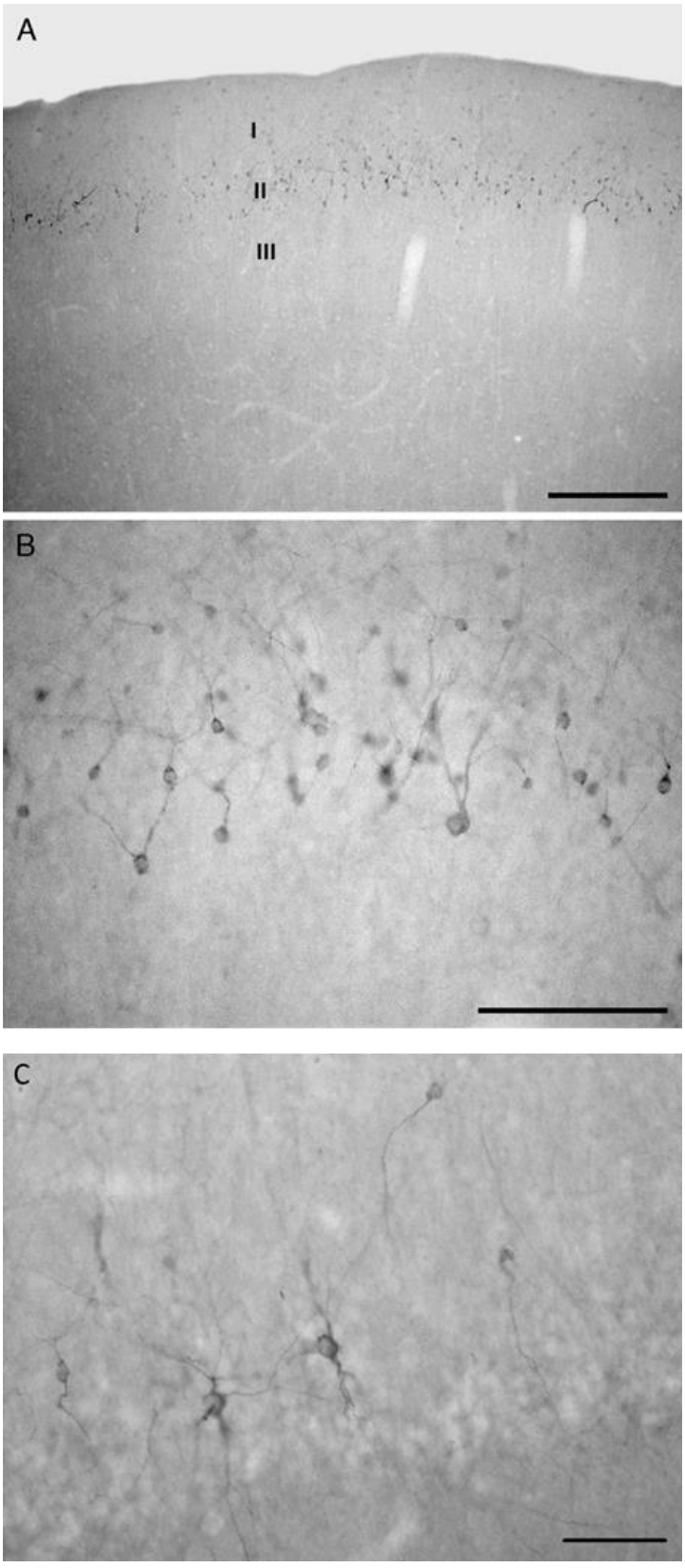
**Figure 4.4**: The mature and immature DCX positive cells in the cerebellum of the Lemur (A and B) at different levels of magnification and in the Galago (C). Scale bar. A= 500 μm; B and C = 50μm.


A
B
C

**Cerebral Cortex**

Addition of neurons in the adult mammalian cerebral cortex has been a controversial issue with some studies reporting neurogenesis and with some not detecting neurogenesis. In this study DCX positive neurons were observed in the various areas of the cerebral cortex in all the species. This positive neurons were observed the second layer of the cerebral cortex (outer granular layer) (see Figure 4.5A) with their dendrites directed towards the outer molecular layer. Majority of DCX positive cell in the cerebral cortex of the Lemur (Figure 4.5B), Galago and Potto were of immature nature because their dendrites showed less extensive branching. The neurons were observed in the frontal part of the cortex up to the primary motor cortex with one or two cells scantly spreading to a part of the visual cortex and became more densely distributed in the piriform cortex (Figure 4.5C).

**Figure 4.5**: Positive DCX cells in the cerebral cortex of the Lemur (A &B) and in the piriform cortex of the Galago (C). Photomicrograph A is showing the different layers of the cerebral cortex namely: I = Molecular layer; II = outer granular layer and III = outer pyramidal layer. Photomicrograph B and C showing cells in higher magnification. Scale Bar= A=250µm and B & C = 100µm.

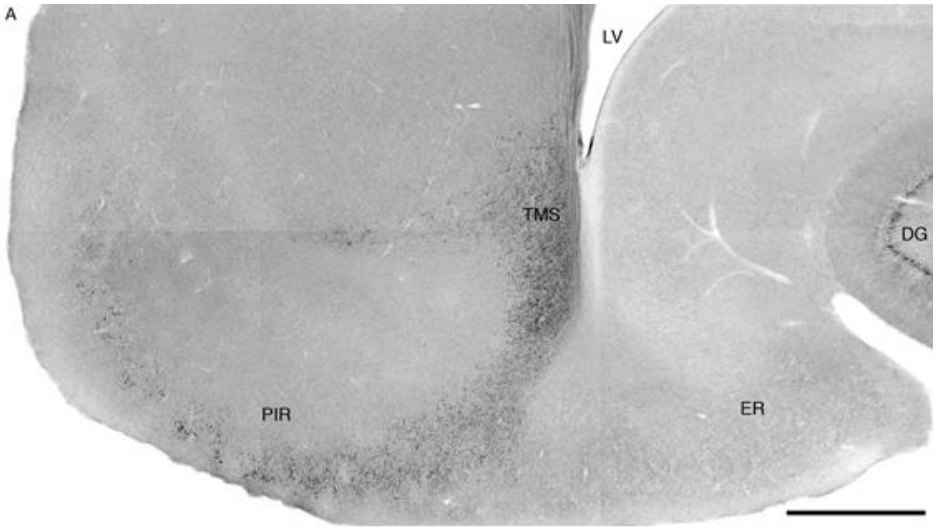

A
I
II
III
B
C

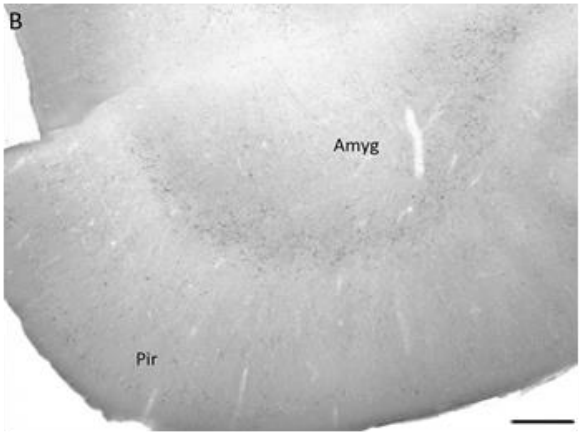
**Piriform cortex, Amygdala and the temporal migratory stream**

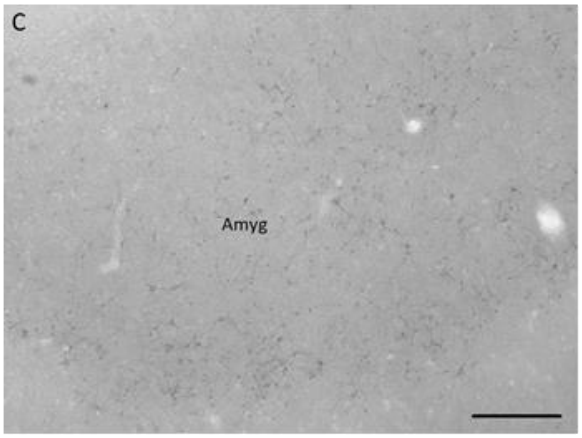
The DCX positive cells were migrating along the temporal migratory stream (TMS) from the SVZ of the lateral ventricle of the temporal horn to the piriform cortex. The temporal migratory stream (TMS) appears to supply cells in the piriform cortex, and the cells observed were mostly unipolar and bipolar neurons (Figure 4.5C), and were migrating in chains in to the second layer of the piriform cortex (Figure 4.6A). This secondary route was found in all the investigated species (Figure 4.6A & B) with abundant immunopositive DCX cells observed in the Lemur.

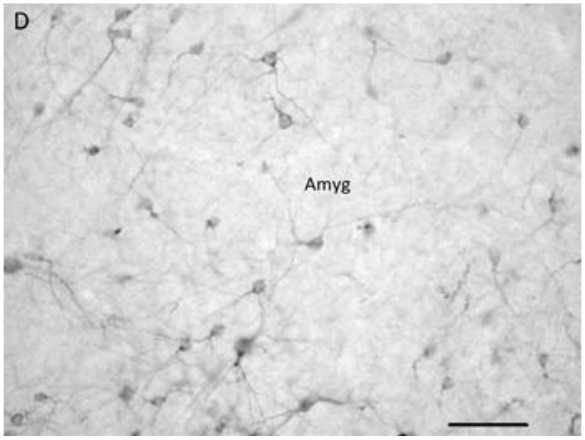
DCX positive cells were observed in the Amygdala of the investigated species. In the Galago (Figure 4.6 B & C) and Potto, most of the DCX positive cell were unipolar and bipolar with few of the cells being multipolar. Their dendrites were dispersed in different directions (Figure 4.6 D).

**Figure 4.6**: Photomicrographs showing DCX positive cells in A: the piriform cortex migrating along the temporal migratory stream (TMS) from the SVZ in the Lemur; B = cells in the piriform cortex and amygdala of the Galago, C = DCX positive cells in the amygdala of the Galago and D =higher magnification of positive cells in the amygdala of the Galago. Amyg = amygdala, TMS=Temporal migratory stream, PIR= piriform cortex. Scale bar: A = 1mm; B & C = 500µm; D=50µm.


A
LV
TMS
DG
PIR
ER


B
Amyg
Pir

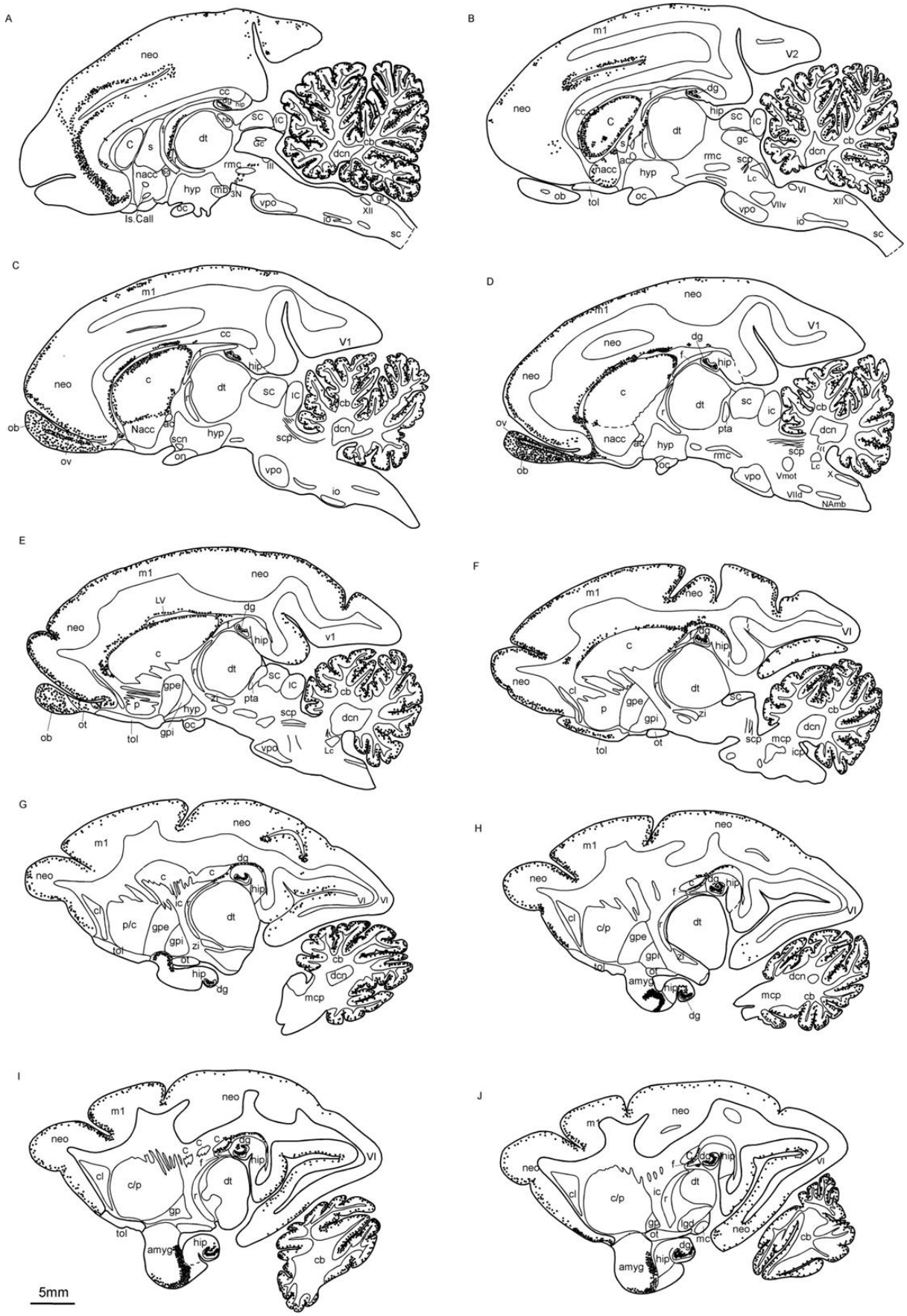

C
Amyg


D
Amyg

**Striatum**

Positive DCX cells were observed in the striatum of all investigated species, with most cells densely distributed in and around the caudate nucleus (Figure 4.7 B, C, and D). Few positive cells were observed spreading to the putamen (Figure 4.7 N) with one or two cells in the nucleus accumbens and claustrum (Figure .4.7 L).

**Figure 4.7**: Schematic diagram showing DCX positive cells in various areas of the brain of the Lemur. The brain was cut into 50 thick sagittal sections with A being the most medial section and T being most the lateral section. The black dot (·) represent the DCX immunoreactive neurons. **III** = Oculomotor nucleus, **IV** = Trochlear nucleus, **Vmot** = motor division of trigeminal nerve nucleus, **VI** = Abducens nucleus, **VIId** = Dorsal division of facial nerve nucleus, **VIIv** = Ventral division of facial nerve nucleus, **X** = Dorsal motor division of Vagus nucleus, **XII** = Hypoglossal nucleus, **3N** = Oculomotor nerve, **M1** = Primary motor cortex, **V1** = Primary visual cortex, **ac** = Anterior Commissure, **Amyg** = Amygdala , **C** = Caudate, **cc** = Corpus callosum, **Cer** = Cerebellum, **Cl** = Claustrum, **DCN** = Deep cerebellar nuclei, **DCX**= Doublecortin, **DG** = Dentate Gyrus , **DT** = Dorsal thalamus, **f** = Fornix, **GC** = Central gray matter of the midbrain, **GP** = Globus pallidus, **Gr** = Gracile nucleus, **Hb** = Habenular, **Hip** = Hippocampus, **Hyp** = Hypothalamus, **ic** = Internal capsule, **IC** = Inferior colliculus, **ICP** = Inferior cerebellar peduncle, **Io** = inferior olivary nucleus, **Lc** = Locus coerulus, **Lgd** = dorsal lateral geniculate nucleus, **Lgv** = ventral lateral geniculate nucleus, **LV** = lateral ventricle, **MB** = Mammilary bodies, **Mc** = main cluster of orexinergic neurons, **mcp** = Middle cerebellar peduncle, **Neo** = Cerebral Neo-Cortex, **N.Acc** = Nucleus Accumbens, **N.Amb** = Nucleus Ambiguus, **OB** = Olfactory Bulb, **OC** = Optic chiasma, **ON** = Optic nerve , **OT** = Optic tract, **OV** = Olfactory ventricle, **P** = Putamen nucleus , **Pir** = Piriform Cortex, **PTa** = Pretectal area, **R** = reticular nucleus of the dorsal thalamus, **Rmc** = magnocellular division of red nucleus, **RMS** = Rostral Migratory Stream , **S** = Septal nuclear complex, **SC** = Superior colliculus, **SCN** = Suprachiasmatic nucleus, **SCP** = Superior cerebellar peduncle, **TOL** = Olfactory tubercle , **Str** = Striatum, **SVZ** = Subventricular zone, **TMS** = Temporal Migratory Stream, **VPO** = Ventral pontine nucleus, **WM** = white matter of the cerebral cortex, **Zi** = Zona incerta.


A
neo
cc
dg
hip
sc
ic
C
s
dt
Gc
cb
dcn
nacc
rmc
III
hyp
mb
3N
vpo
gl
XII
oc
io
sc
Is.Call
B
m1
V2
neo
cc
C
dg
hip
sc
ic
dt
gc
cb
dcn
s
r
ac
nacc
hyp
rmc
scp
Lc
VI
XII
ob
tol
oc
VIIv
vpo
io
sc
C
m1
cc
V1
neo
c
hip
dt
sc
ic
cb
ob
Nacc
ac
scn
hyp
scp
dcn
ov
on
vpo
io
D
neo
m1
V1
neo
dg
hip
neo
c
dt
sc
ic
cb
ov
nacc
ac
hyp
pta
dcn
rmc
scp
Lc
ob
oc
vpo
Vmot
X
VIId
NAmb
E
m1
neo
LV
dg
neo
hip
v1
c
dt
sc
ic
gpe
pta
cb
p
hyp
zi
scp
dcn
ob
ot
tol
gpi
oc
vpo
Lc
F
m1
neo
VI
c
dg
hip
neo
cl
dt
sc
p
gpe
gpi
zi
cb
dcn
tol
ot
scp
mcp
icp
G
neo
m1
dg
neo
c
c
hip
ic
VI
VI
cl
p/c
gpe
dt
gpi
zi
tol
ot
cb
hip
dcn
dg
mcp
H
neo
m1
neo
f
c
dg
hip
VI
cl
c/p
dt
gpe
gpi
zi
tol
ot
amyg
hip
dcn
mcp
cb
dg
I
neo
m1
neo
c
c
c
dg
f
hip
VI
cl
c/p
dt
r
gp
tol
amyg
hip
cb
5mm
J
neo
m1
neo
f
dg
hip
VI
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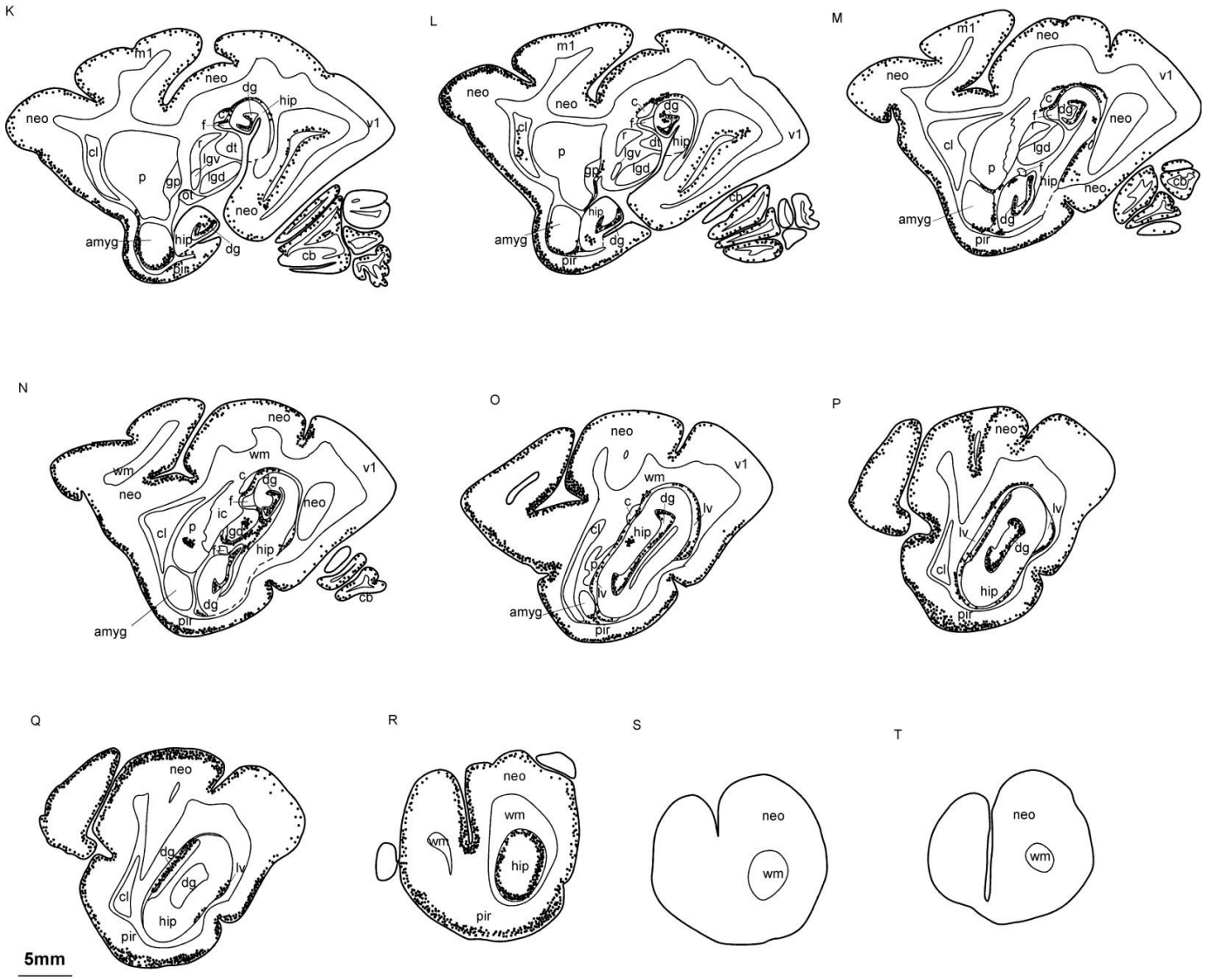
ot
mc
neo
cb
dg
amyg
hip


K
m1
neo
dg
hip
v1
f
dt
lgv
lgd
cl
p
gp
ot
amyg
pir
cb
L
M
N
wm
ic
O
lv
P
Q
R
S
T
5mm

**Pattern of DCX positive cell distribution of cells**

Nissl stain was used in the diagrammatic reconstruction of a series of sagittal sections from the brain of the Lemur. The immunohistochemically treated sections were used to mark the location of immunopositive neurons. These drawings illustrate the location of neurons that were immunohistochemically reactive for DCX. Figure 4.7 is showing the drawings of the different sections of the brain where A represent the most medial section and T represent the most lateral section.

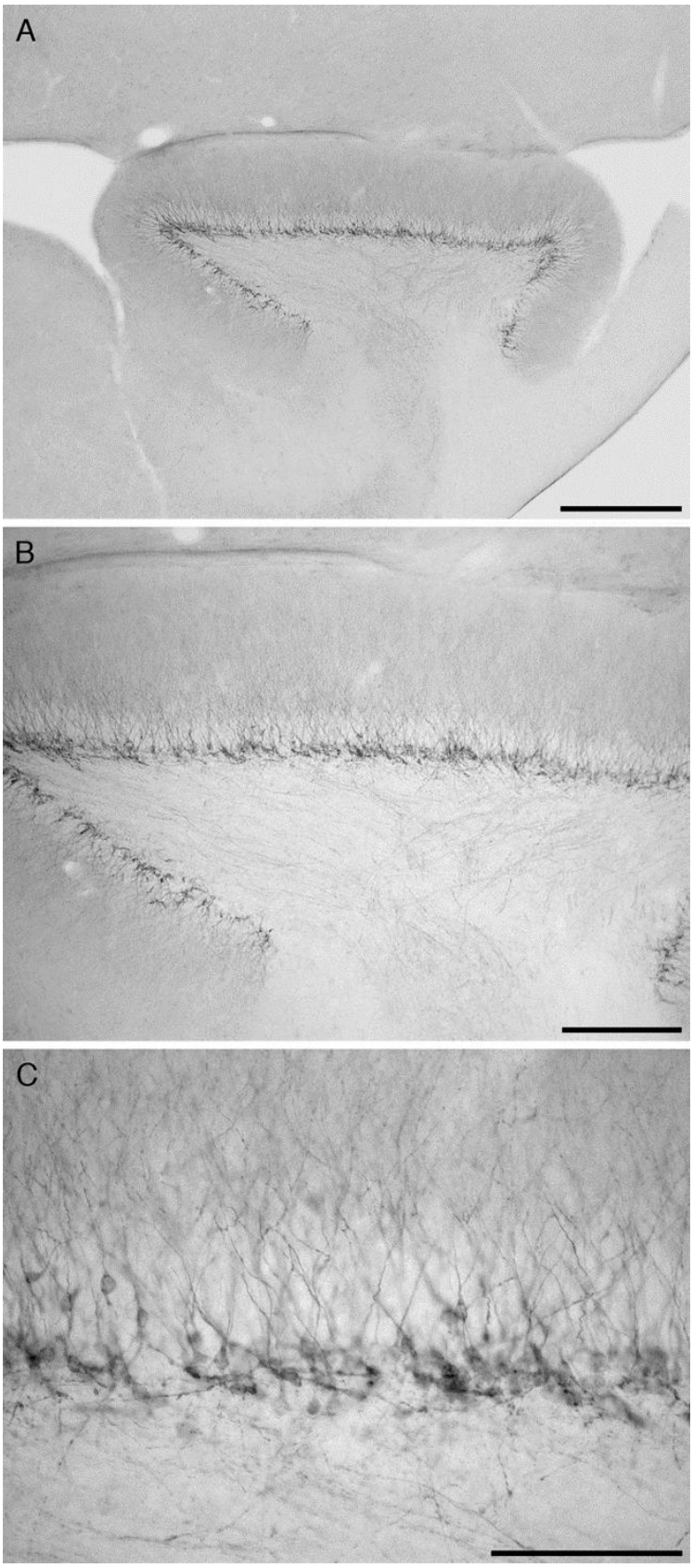
Figure A illustrated how the DCX immunopositive neurons were migrating from the SVZ of the lateral ventricle to the various parts of the cerebral cortex. Figure A to N illustrated how cells were distributed in the cerebellum. Figure A to R showed cells in the dentate gyrus and few spreading in the hippocampus. Figure B to R showed the immunopositive neurons in different regions of the cerebral cortex. Figure B to D illustrated the cells in the striatum with most of cells distributed in the caudate nucleus and some few cells in the nucleus accumbens with few other cells spreading to the septum. Figure C to E illustrated cells observed in the SVZ and were moving along RMS in chains to the OB. Figure G to H illustrated cells that were distributed in the temporal horn of the lateral ventricle, along the temporal migratory stream and amygdala. Figure K to R illustrated cells in the piriform cortex.

### 4.3 Categorization of DCX positive cells in the dentate gyrus (DG) of the three investigated species.

The morphology of the newly generated cells as revealed by the DCX immunohistochemistry in the dentate gyrus was categorized according to the study by Plumpe and colleagues (2006). The immature neurons were observed to send their dendrites towards the molecular layer of the dentate gyrus.

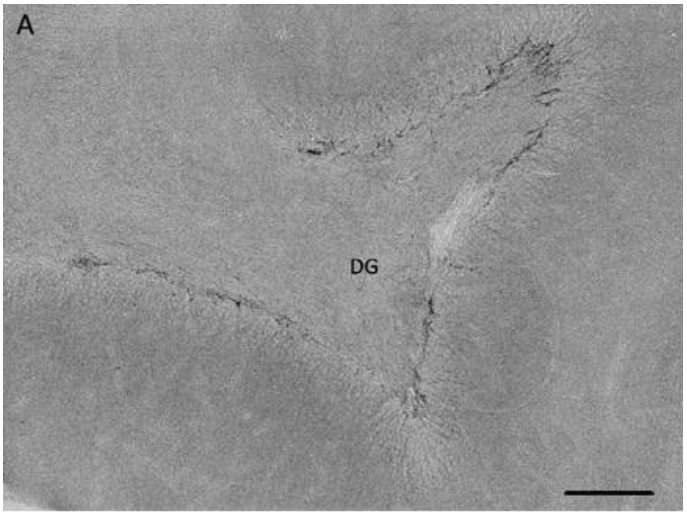
In the dentate gyrus of the Lemur, the photomicrographs at different levels of magnification (Figure 4.8 A to C) showed that majority of the DCX positive cells were classified under category F (Plumpe et al., 2006), because these cells were of mature appearance and had their dendrites reaching the molecular layer and showed dense branching within the granule cell layer (Figure 4.8C). Their cell bodies were embedded in the sub-granular zone layer and with some extending their axonal projections toward the hippocampal CA3 pyramidal cell layer. Few bipolar neurons were also visible.

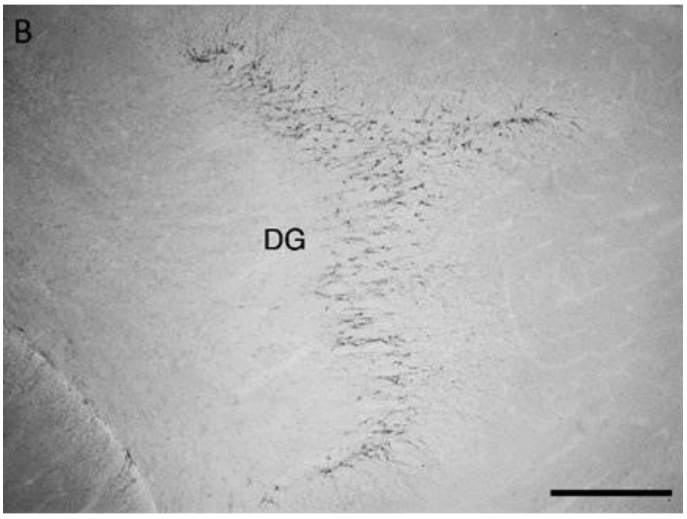
**Figure 4.8**: Positive DCX cells in the DG of the Lemur at different levels of magnification. Scale bar: A = 500µm, B = 250µm and C = 100µm.

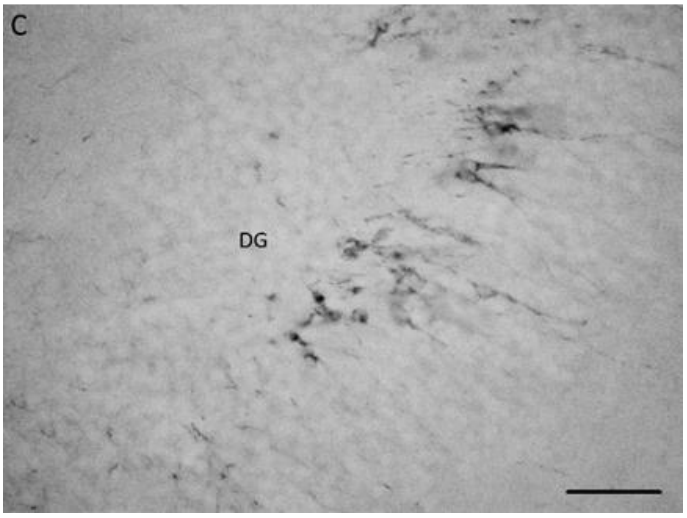

A
B
C

In the dentate gyrus of Potto the photomicrographs at different levels of magnification (Figure 4.9A to C) showed that most of the DCX positive cells were classified under category E as they had one dendrite penetrating the molecular layer with sparse dendritic branching (Figure 4.9C). Few immature cells with no processes (category A) and immature cells with very short processes (category B) were observed in this species. There were one or two cells that were classified under category F visible.

**Figure 4.9**: Representative photomicrographs showing DCX positive cells in the dentate gyrus of Potto at different levels of magnification. Scale bar: A= 500µm, B = 250µm and C = 100µm.

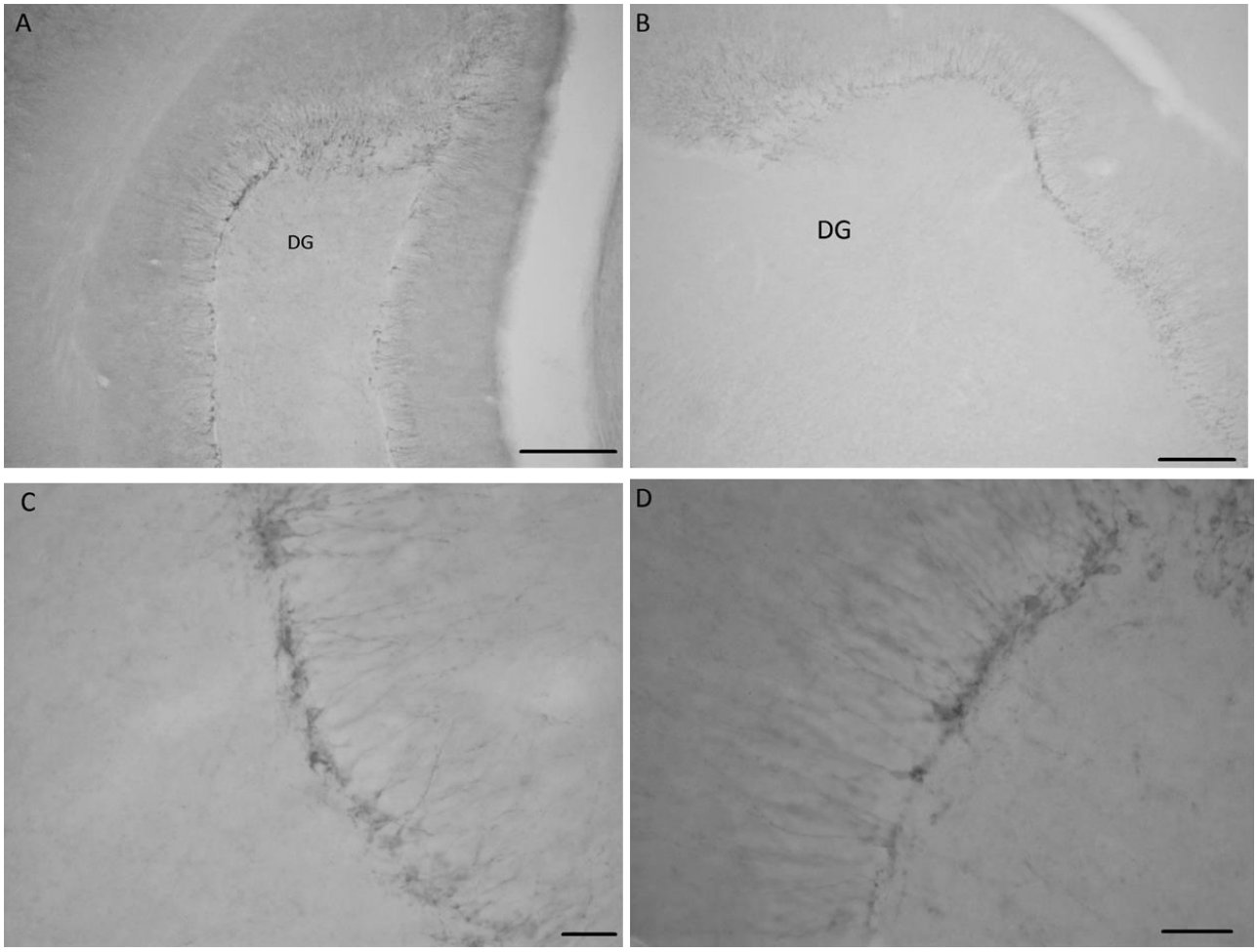

A
DG


B
DG


C
DG

The DCX positive cells in the dentate gyrus of the Galago at different levels of magnification (Figure 4.10 A – D) showed that most of the DCX cells were falling under category E because the cells were of mature nature; they had one dendrite penetrating the molecular layer and showed sparse dendritic branching (Figure 4.10C). Some few positive cells observed were classified under category F because the cells showed a mature appearance with their dendrite reaching the molecular layer (Figure 4.10 C and D) and showing dense branching within the granule cell layer. Fewer immature cells falling under category A (with no processes) and B (with very short processes) were visible (Figure 4.10D).

**Figure 4.10**: Photomicrographs showing the DCX positive cells in the Galago. Scale bar A & B = 500µm and C & D = 100µm.


A
DG
B
DG
C
D

In summary using the Doublecortin as a marker of immature neurons, cell proliferation was observed in active neurogenic sites and potential sites. A summary of the results of the density and distribution of DCX positive cells in various areas of the brain in the three prosimian primates is presented in Table 4.1.

**Table 4.1**: Summary of results showing the sites and density of DCX positive cells

| Name | SVZ | DG | OB | RMS | CC | Neo | Pir | Amyg | TMS | Str | Cer |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lemur** | +++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ | ++ | ++ | +++ |
| **Galago** | + | + | + | + | + | + | + | + | + | + | + |
| **Potto** | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | + | - |

SVZ = Subventricular zone; DG = Dentate Gyrus; OB = Olfactory Bulb; RMS = Rostral Migratory Stream; CC = Corpus callosum; Neo= Neo-Cortex; Pir = Piriform Cortex; Amyg = Amygdala; TMS = Temporal Migratory Stream; Str = Striatum; Cer = Cerebellum (+++) as high density; (++) as moderate density; (+) low density; (-) as no cells detected.

## 4.4 Results on Ki-67

### 4.4.1 Ki-67 cells on established active neurogenic sites

**Sub-ventricular zone of the lateral ventricle and the Olfactory bulb**

Proliferating cells were observed in the SVZ of the lateral ventricle and were running along the rostral migratory stream (RMS) to the olfactory bulb (OB) in all the three investigated species. The proliferating cells were observed along the rostral migratory stream (RMS) (Figure 4.11A) and were migrating from the SVZ (Figure 4.11B) to the granule cell layer of the OB. Figure 4.12 A and B showed the Ki-67 proliferating cells in the olfactory bulb of the Galago and Potto respectively. Figure 4.12 C and D showed the cells in higher magnification, the arrow in Figure 4.12 D showed a dividing cell, suggesting a continuous cell division. Potto had the highest density of proliferative cells in the olfactory bulb compared to the two other species.

**Figure 4.11**: Photomicrographs showing Ki-67 cells in various areas of the brain of the Lemur. Photomicrograph A showed positive cells moving along the rostral migratory stream (RMS) while photomicrograph B showed the positive cells on the SVZ of the lateral ventricle (LV). Scale bar: A = 500 µm and B = 250 µm.

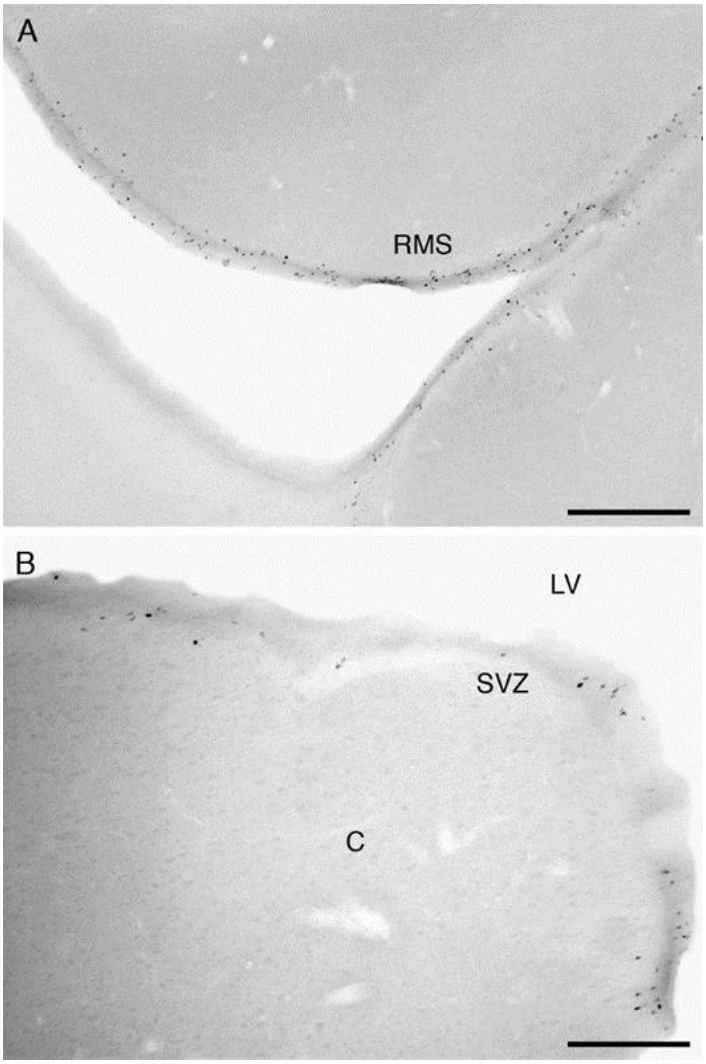
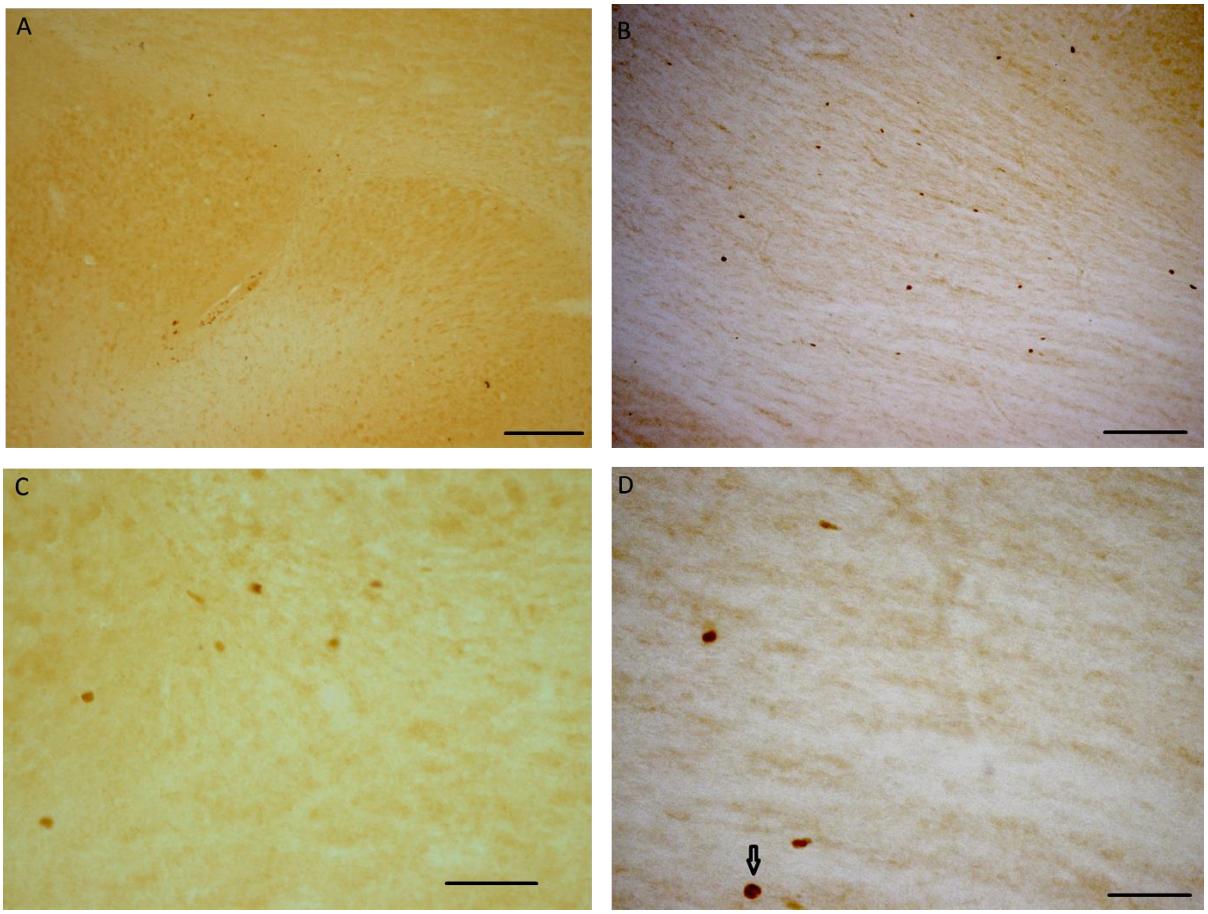

A
RMS
B
LV
SVZ
C

**Figure 4.12**: Photomicrographs showing Ki-67 cells in the olfactory bulb (OB) of the Galago (A & C) and Potto (B & D). Photomicrographs A and B showed cells in medium power and C and D showed cells in higher magnification. The arrow showed a dividing cell. Scale bar: A & B = 500µm; C & D = 100µm.


A
B
C
D

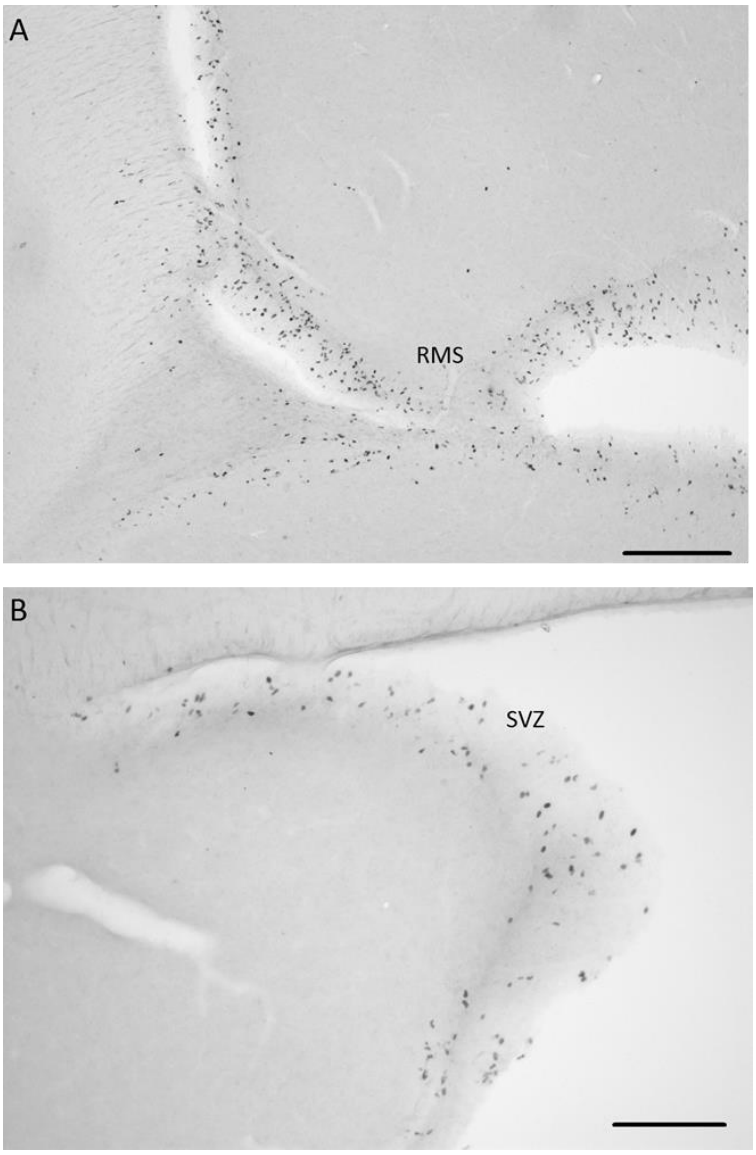
When comparing the cell distribution within the three species, Potto had densely distributed cells in the olfactory bulb ( Figure 4.12 B & D), the rostral migratory stream (Figure 4.13A) and the Subventricular zone (Figure 4.13 B) compared to the other two species. Majority of cells along the rostral migratory stream in Potto (Figure 4.13 A) were in pairs and diving faster than the cells in the Ring tailed lemur (Figure 4.11 A) and Galago (Figure 4.14B).The Galago (Figure 4.14) had more scantly distributed cells compared to the other two species.

**The rostral migratory stream and temporal migratory stream**

In all the species investigated the rostral migratory stream was observed. The density of the Ki-67 proliferative cells differed in each animal. The Potto species had the highest density of cells with some of the cells spreading to the neighbouring structures of the pathway (Figure 4.13 A). Both the ventral and the dorsal limb of the RMS as seen in Figure 4.13 A were thicker and had abundant paired cells with large and oval shape nuclei.

In the Lemur (Figure 4.11 A) and the Galago the RMS was made up of single round cells which were more scantly distributed in the Galago (Figure 4.14 B) The temporal migratory stream is a secondary pathway that is strongly observed in the primate species, however in these three species the Ki- 67 positive cells were observed more in the Potto with few cell in the Lemur and none in the Galago.

**Figure 4.13**: Photomicrograph showing Ki-67 positive cells various areas of the brain of the Potto. Photomicrograph A showed cells in the rostral migratory stream and photomicrograph B showed cells in the subventricular zone. Scale bar: A= 500µm; B = 250µm.


A
RMS
B
SVZ

## The dentate gyrus of the hippocampus

Proliferative cells were observed in the dentate gyrus of all the investigated species. The results in Figure 4.14 A showed scantly distributed Ki-67 proliferative cells in the dentate gyrus of the Galago. The results in Figure 4.15 showed the Ki-67 proliferating cells in the dentate gyrus of the Lemur at different levels of magnification. More densely distributed and intensely stained proliferative cells were observed in the dentate gyrus of the Potto (Figure 4.16).

Interspecies comparison indicated that the Ki-67 proliferating cells were more densely distributed in Potto compared to the other two species. Majority of the Ki-67 proliferating cells were found in pairs or clusters of four as indicated with the arrows in Figure 4.16 B, suggesting an extensive cell division. In the Galago, majority of cells were single (Figure 4.14 A) while in the Lemur some cell were single (Figure 4.15 A) with few cells in pairs (Figure 4.15 B).

In the Galago, these nuclei were small and rounded, while in the Lemur most of the nuclei were also round and small with some few cells having an oval shape. Majority of the nuclei in Potto were large and oval shaped with few round and small nuclei (Figure 4.16 C).

**Figure 4.14**: Photomicrographs showing Ki-67 positive cells in the Galago Photomicrograph A showed ki-67 positive cells in the dentate gyrus and photomicrograph B showed Ki-67 positive cells in the rostral migratory stream. Scale bar: A & B = 500µm

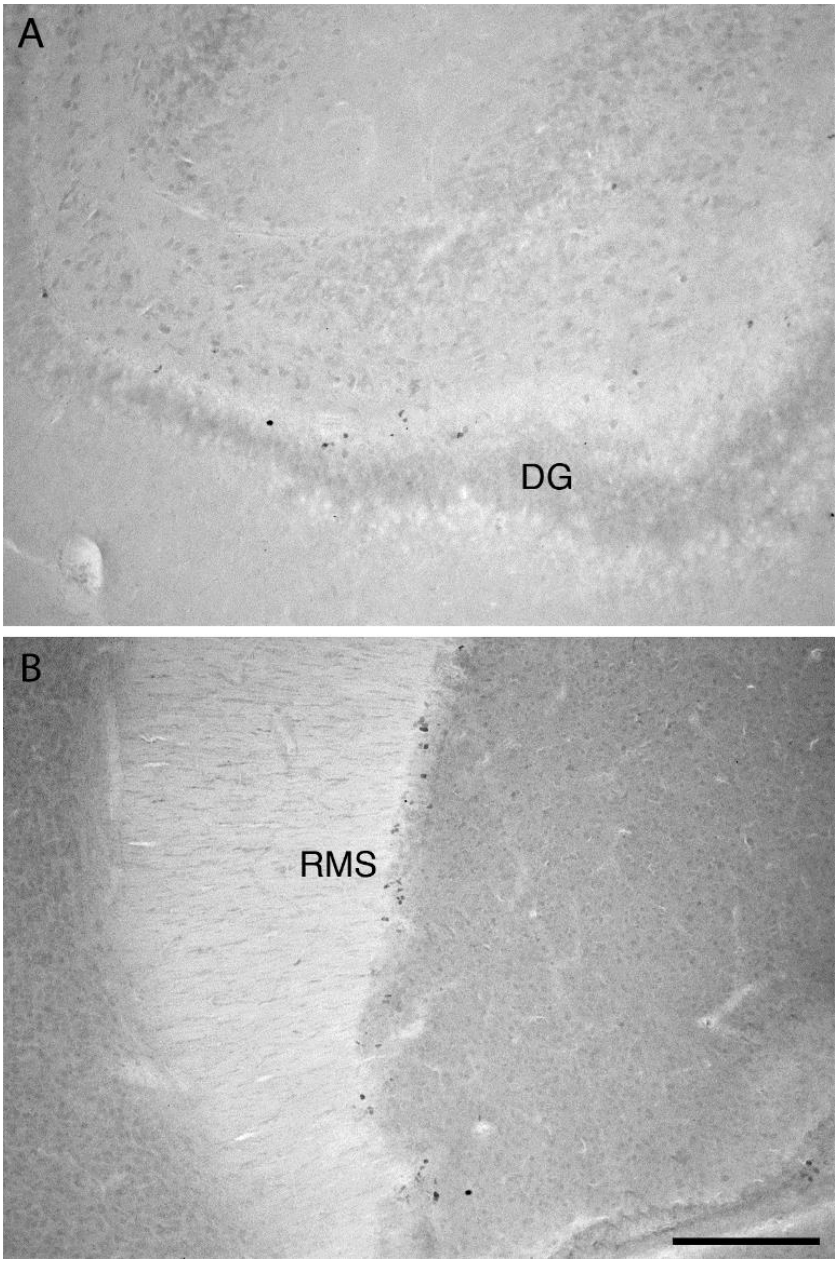

A
DG
B
RMS

**Figure 4.15**: Positive Ki-67 in the dentate gyrus (DG) of the Lemur photomicrograph A showed cells in the medium power and photomicrograph B showed cells in higher magnification. Scale bar. A = 250µm; B = 100µm.

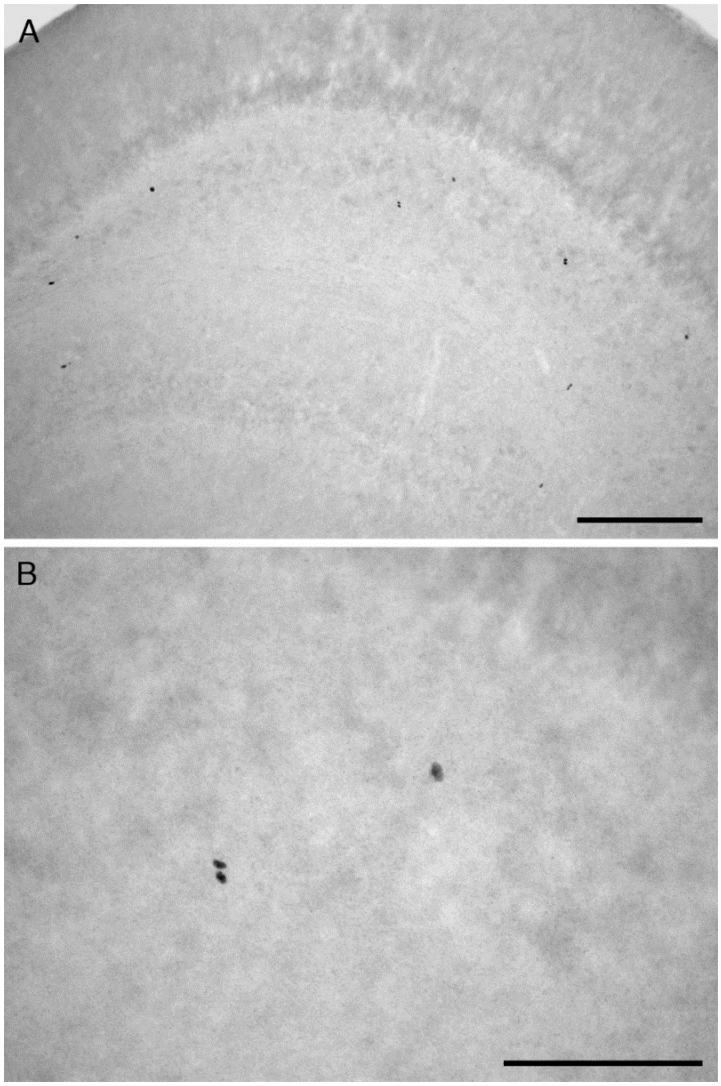
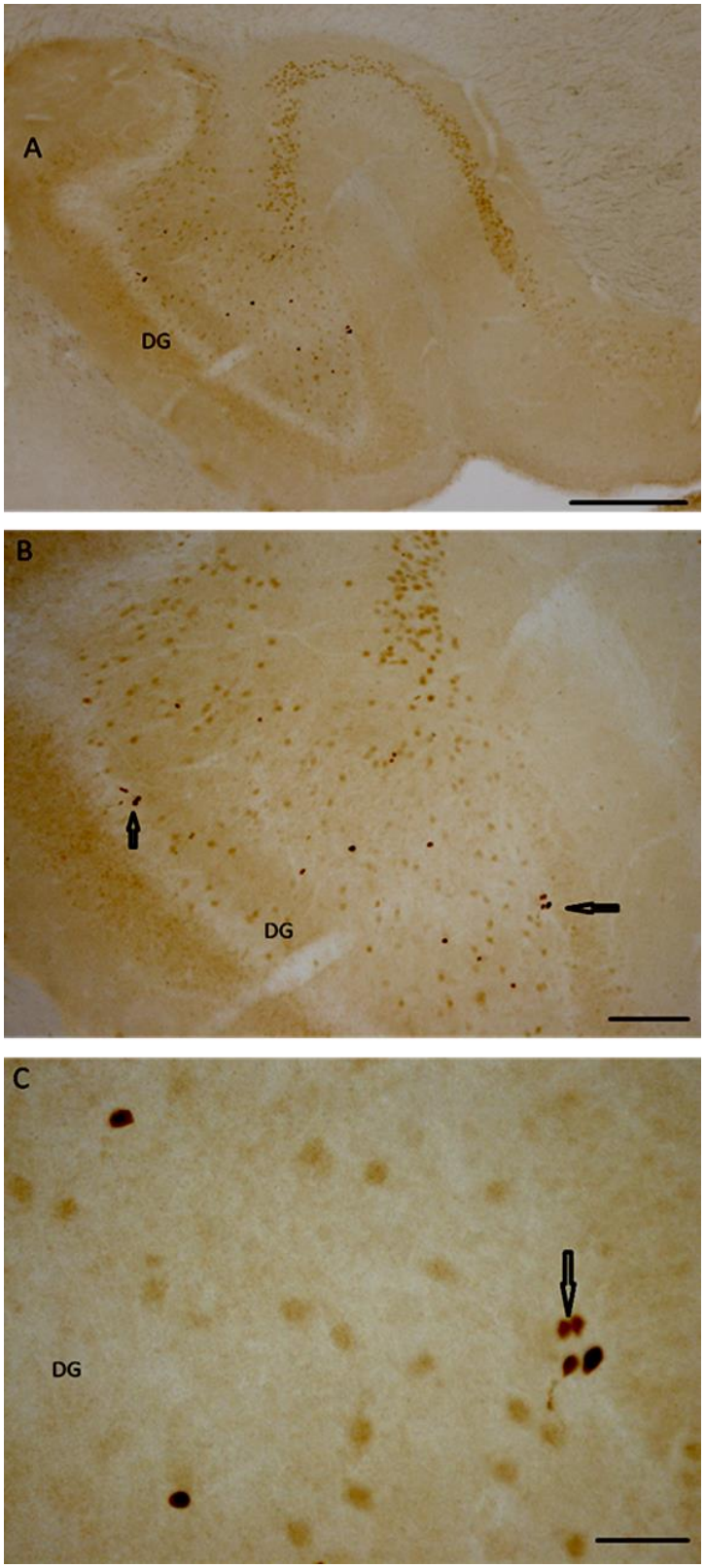

A
B

**Figure 4.16**: Photomicrograph showing Ki-67 cells in the dentate gyrus (DG) of the Potto at different levels of magnification. Photomicrograph A showing cells in low power, B showing cells in the medium power and C showing cells in high power. Scale bar: A = 500µm; B = 250µm and C = 100µm


A
DG
B
DG
C
DG

### 4.4.2 Ki-67 in Potential sites

**Piriform cortex and Amygdala**

Ki-67 proliferating cells were observed in the amygdala of the Lemur and the Potto, whereas in the Galago no Ki-67 proliferative cell were observed in the amygdala. Fewer Ki-67 proliferative cells were observed in the Lemur compared to the Potto. In the piriform cortex few Ki-67 immunopositive cells were observed only in the Potto with none observed in the Lemur and the Galago.

**Striatum**

Ki-67 proliferative cells were observed in the potential sites such as striatum and few cells in the corpus callosum in all the species. Ki-67 proliferative cells were observed in the striatum with more cells densely distributed in and around the caudate nucleus and few cells in the Nucleus accumbens ( Figure 4.18C) and putamen with one or two cells spreading to the claustrum (See illustration in figure 4.18 A to Q). However in the Galago no cells were observed in the caudate nucleus only few scantly distributed Ki-67 cells were observed around the caudate nucleus.

**Cerebellum and Cerebral cortex**

In this study no Ki-67 proliferative cells were observed in the cerebellum (See Figure 4.17A) and cerebral cortex in all the investigated species (see Figure 4.17B).

**Figure 4.17**: Photomicrographs showing that no Ki-67 positive cells were observed in the cerebellum (A) and the cerebral cortex (B). Scale bar = 500µm

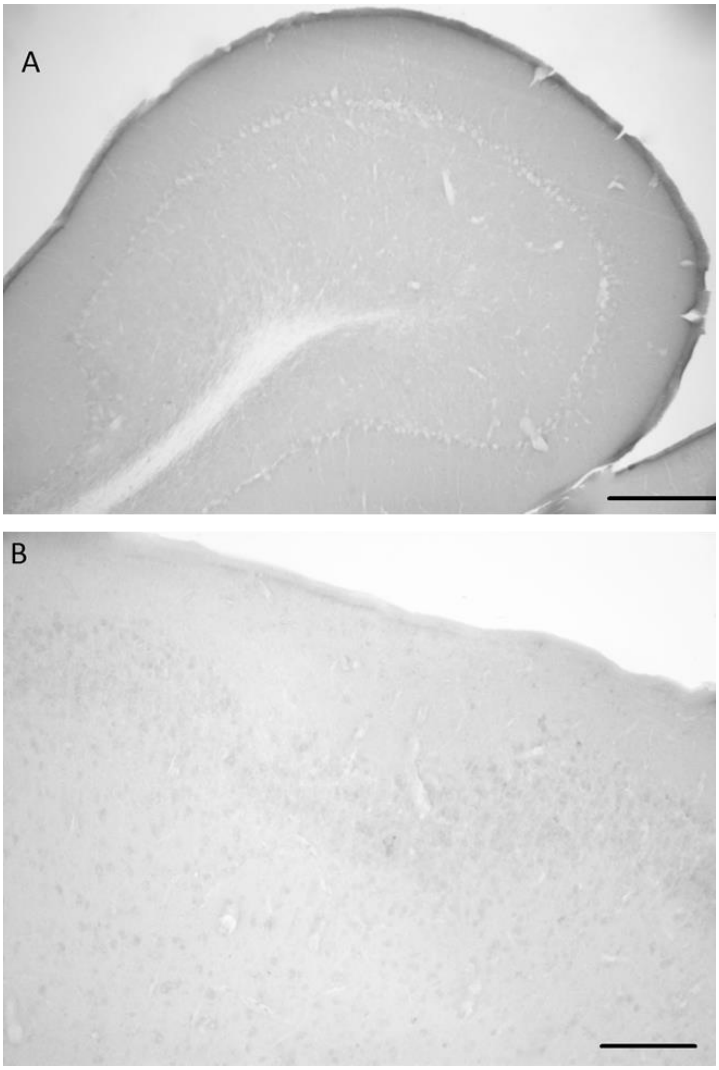

A
B

**Table 4.2:** Qualitative Summary showing sites and density of Ki-67 in various regions of the prosimians brains

| Name | SVZ | DG | OB | RMS | C | CC | Neo | Pir | Amyg | TMS | Str | Cer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lemur** | +++ | +++ | ++ | ++ | + | ++ | - | - | + | + | + | - |
| **Galago** | + | + | + | + | - | + | - | - | - | - | + | - |
| **Potto** | ++++ | ++++ | +++ | +++ | ++ | +++ | - | + | ++ | ++ | ++ | - |

SVZ = Subventricular zone; DG = Dentate Gyrus; OB = Olfactory Bulb; RMS = Rostral Migratory Stream; C = Caudate; CC = Corpus callosum; Neo= Neo-Cortex; Pir = Piriform Cortex; Amyg = Amygdala; TMS = Temporal Migratory Stream; Str = Striatum; Cer = Cerebellum; (+++) as high density; (++) as moderate density; (+) low density; (-) as no cells detected.

**Figure 4.18**: Schematic diagrams illustrating the distribution of Ki-67 positive cells in various areas of the brain of the Ring tailed Lemur. The Brain was cut into 50µm thick sagittal sections with A being the most medial section and T being the most lateral section. The black dot (·) represented the Ki-67 immunopositive cells. **III** = Oculomotor nucleus, **IV** = Trochlear nucleus, **Vmot** = motor division of trigeminal nerve nucleus, **VI** = Abducens nucleus, **VIId** = Dorsal division of facial nerve nucleus, **VIIv** = Ventral division of facial nerve nucleus, **X** = Dorsal motor division of vagus nucleus, **XII** = Hypoglossal nucleus, **3N** = Oculomotor nerve, **M1** = Primary motor cortex, **V1** = Primary visual cortex, **ac** = Anterior Commissure, **Amyg** = Amygdala , **C** = Caudate, **cc** = Corpus callosum, **Cer** = Cerebellum, **Cl** = Claustrum, **DCN** = Deep cerebellar nuclei, **DCX**= Doublecortin, **DG** = Dentate Gyrus , **DT** = Dorsal thalamus, **f** = Fornix, **GC** = Central gray matter of the midbrain, **GP** = Globus pallidus, **Gr** = Gracile nucleus, **Hb** = Habenular, **Hip** = Hippocampus, **Hyp** = Hypothalamus, **ic** = Internal capsule, **IC** = Inferior colliculus, **ICP** = Inferior cerebellar peduncle, **Io** = inferior olivary nucleus, **Lc** = Locus coerulus, **Lgd** = dorsal lateral geniculate nucleus, **Lgv** = ventral lateral geniculate nucleus, **LV** = lateral ventricle, **MB** = Mammilary bodies, **Mc** = main cluster of orexinergic neurons, **mcp** = Middle cerebellar peduncle, **Neo** = Cerebral Neo-Cortex, **N.Acc** = Nucleus Accumbens, **N.Amb** = Nucleus Ambiguus, **OB** = Olfactory Bulb, **OC** = Optic chiasma, **ON** = Optic nerve , **OT** = Optic tract, **OV** = Olfactory ventricle, **P** = Putamen nucleus , **Pir** = Piriform Cortex, **PTa** = Pretectal area, **R** = reticular nucleus of the dorsal thalamus, **Rmc** = magnocellular division of red nucleus, **RMS** = Rostral Migratory Stream , **S** = Septal nuclear complex, **SC** = Superior colliculus, **SCN** = Suprachiasmatic nucleus, **SCP** = Superior cerebellar peduncle, **TOL** = Olfactory tubercle , **Str** = Striatum, **SVZ** = Subventricular zone, **TMS** = Temporal Migratory Stream, **VPO** = Ventral pontine nucleus, **WM** = white matter of the cerebral cortex, **Zi** = Zona incerta.

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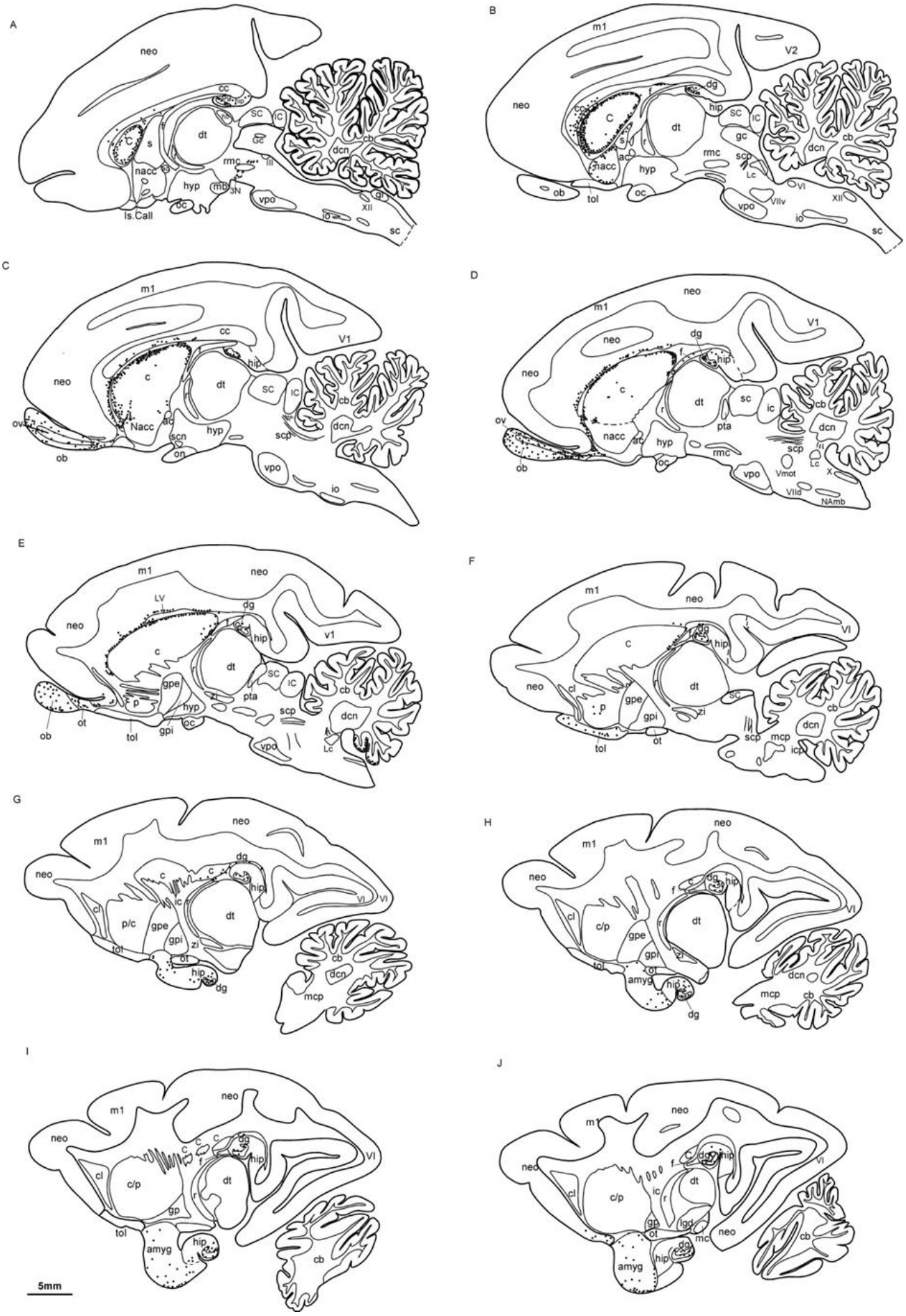
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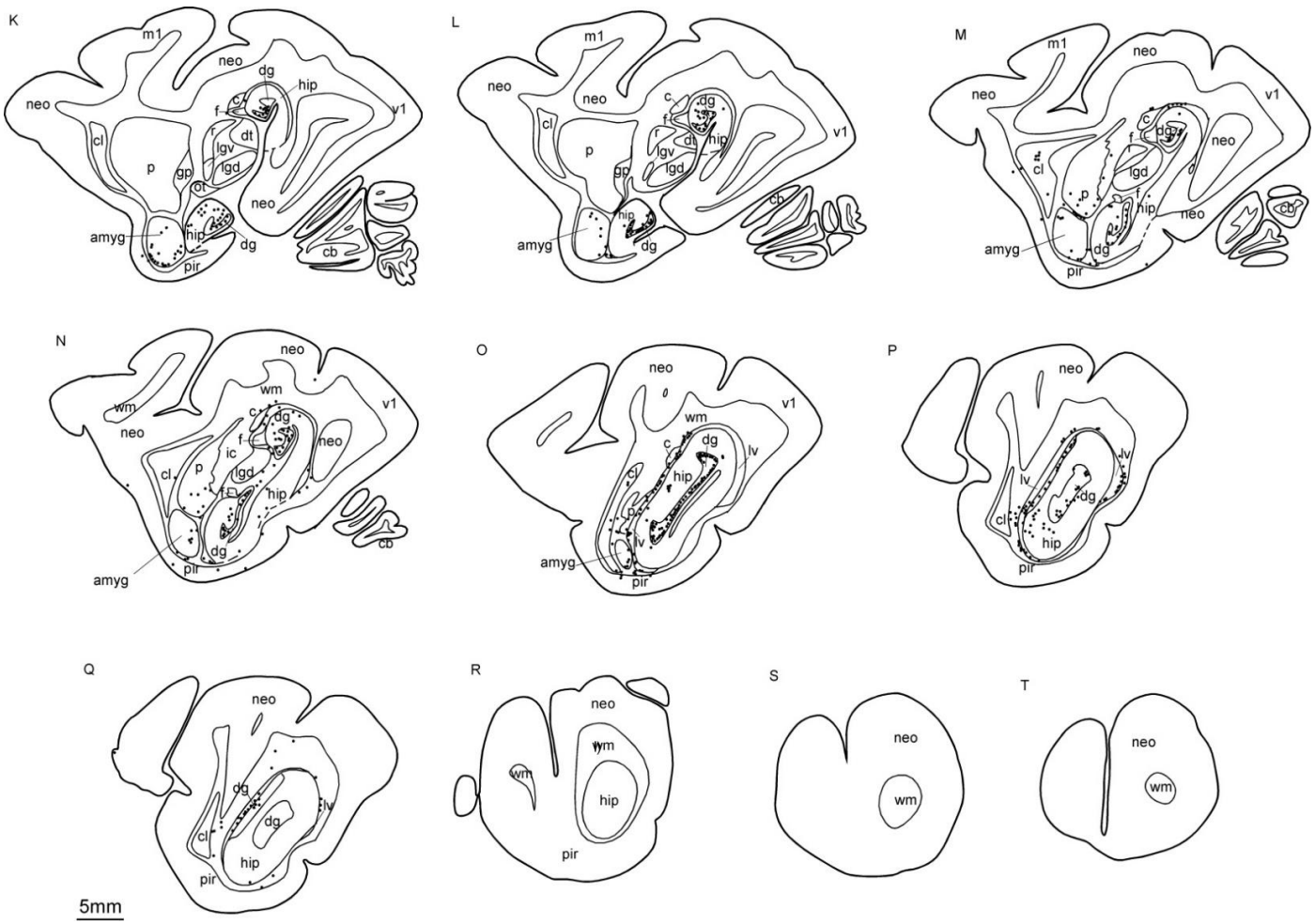
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**Pattern of Ki-67 positive cell distribution**

The schematic drawings in Figure 4.17 (A-T) illustrated the distribution of Ki-67 positive cells at different brain sections in the Lemur. Figure A represents the most medial section and Figure 4.17 T represent the most lateral section. Figure A to T illustrated that no Ki-67 immunopositive neurons were observed in the various parts of the cerebral cortex. Figure B to D illustrated the cells in the striatum with most cells in the caudate nucleus, `few spreading in the nucleus accumbens one or two in the septum. Figure C to E illustrated cells in the SVZ, along the rostral migratory stream and the olfactory bulb. Figure G to J illustrated cells that were distributed in the temporal horn of the lateral ventricle, along the TMS and amygdala. Figure K to R illustrated that no cells were observed in the piriform cortex and Figure A-N illustrate that no cells were observed in the cerebellum.

## 4.5 Proliferating cell number of Ki-67 cells

The rate of cell proliferation in the dentate gyrus of the hippocampus was estimated through the counting of Ki-67 positive cells, and multiplied the total number by the section's sampling fraction to obtain the estimated total proliferating cell number. The left hemisphere of the sagittal sections of all the species was used for counting and only the cells within the SGZ and the GCL were counted. The minimum values obtained as per the counting of Ki- 67 cells per species were: Potto = 4 cells; Lemur = 3 cells and Galago = 2 cells while the maximum value obtained was 59 cells; 35 cells and 27 cells for Potto, Lemur and the Galago respectively. The mean values obtained from this were used for statistical analysis.

The total number of Ki-67 cells was calculated and multiplied by each section sampling fraction of four. This was depicted in a form of a graph in Figure 4.19 and showed that Potto had the highest number of proliferative cells. When comparing within the three species, Potto had 4.8 times higher number of proliferative cells than Galago and 1.9 times higher than the Ring tailed Lemur. The variation of the rate of cell proliferation within the species was statistically determined by unpaired student t-test whereby the differences between the group means was analysed and found to be significantly different when comparing Potto with the Lemur ($p \leq 0.05$), Potto and the Galago ($p \leq 0.05$). However there was no significant difference when the mean values of the Lemur and the Galago were compared ($p \geq 0.05$).

**Figure 4.19**: A graph showing total number of Ki-67 cells in the sagittal sections in the brains of the three prosimian primates.

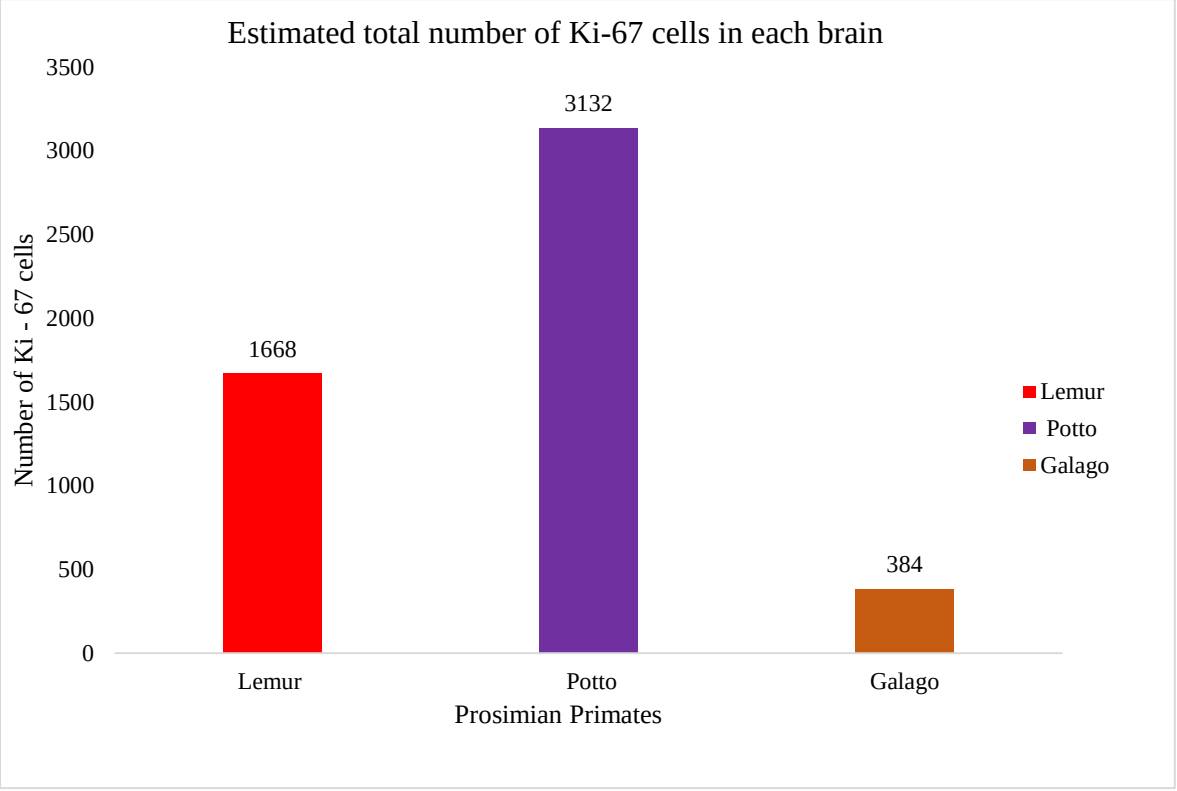

Estimated total number of Ki-67 cells in each brain
Number of Ki - 67 cells
3500
3000
2500
2000
1500
1000
500
0
1668
3132
384
Lemur
Potto
Galago
Prosimian Primates
Lemur
Potto
Galago

**Figure 4. 20**: A graph showing total number of Ki-67 cells in the coronal sections in the brains of the three prosimian primates.

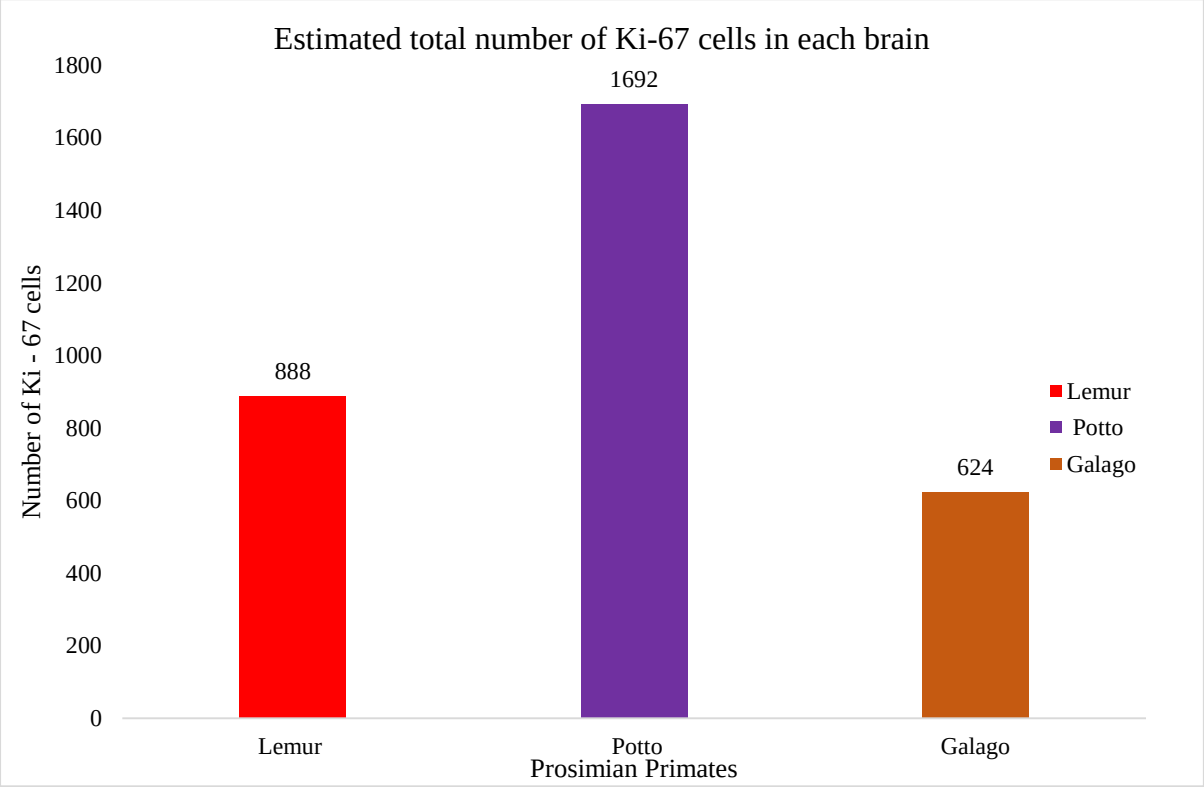
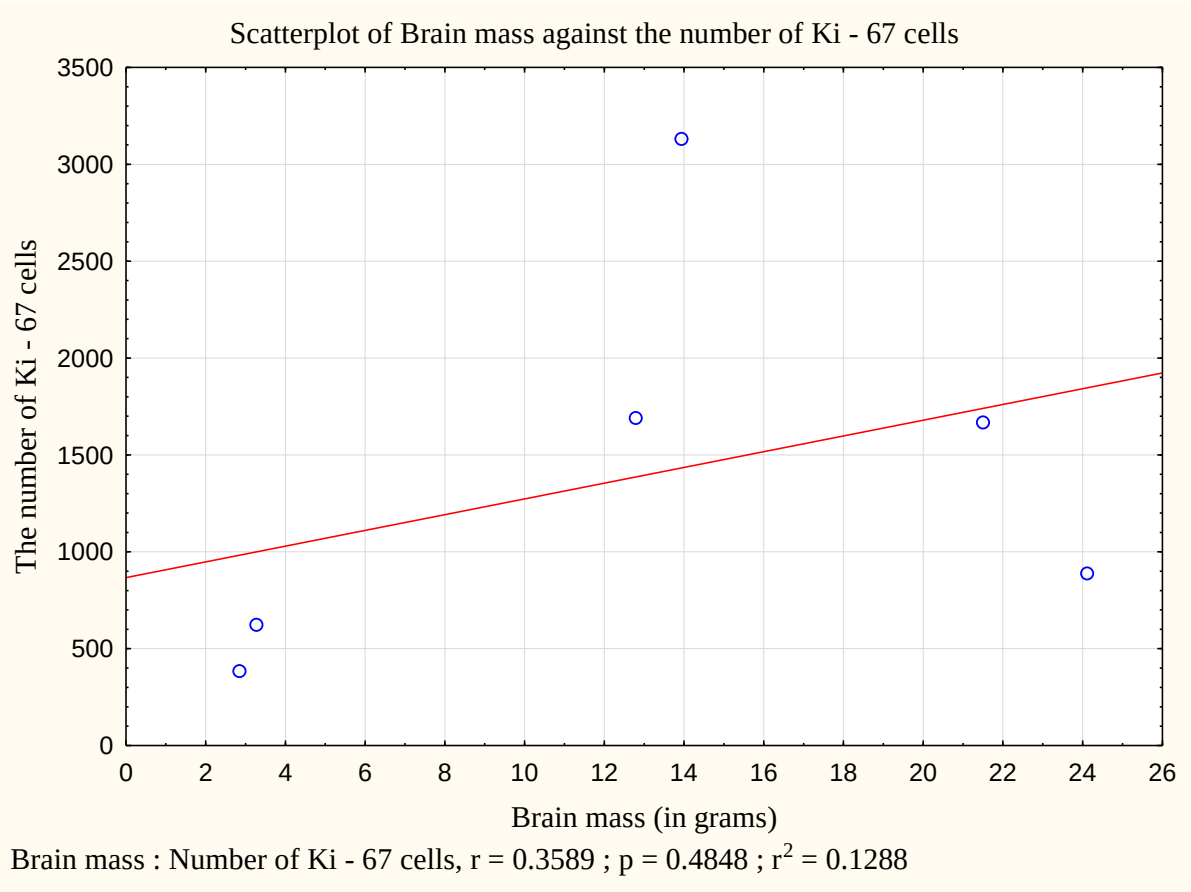

Estimated total number of Ki-67 cells in each brain
Number of Ki - 67 cells
1800
1600
1400
1200
1000
800
600
400
200
0
888
1692
624
Lemur
Potto
Galago
Lemur
Potto
Galago
Prosimian Primates

**Figure 4.21:** A scatter plot graph showing total number of Ki-67 cells against the brain mass of the brains of the three prosimian primates.




Brain mass : Number of Ki - 67 cells, r = 0.3589 ; p = 0.4848 ; $r^2$ = 0.1288

The statistical results proved that there other factors that contributed to the variations in the number of Ki-67 cells within the three species. Therefore a regression analysis was run in order to determine if the brain mass could have contributed to these variations. The results on regression analysis showed that only 13% of variation in Ki67 count was explained by brain Mass ($r^2$ = 0.1288). Besides, the results suggests that if brain mass is increased by 1 unit (1gr), the overall cell count is expected to increase by 41 cell counts (coefficient=40.63, Std. Error =52.84). However, this has been statistically not significant (p-value=0.485).

# Chapter 5: DISCUSSION

The aims of the present study were to investigate *(i)* whether adult neurogenesis occurs in active neurogenic sites *(ii)* immature or maturing neurons are found in potential neurogenic sites *(iii)* the morphology of the newly generated DCX cells and characterize them and *(iv)* estimate the rate of cell proliferation in the dentate gyrus of the hippocampus through the counting of Ki-67 positive cells. The current study confirmed the presence of Ki-67 proliferating cells and newly generated DCX neurons were observed in the established active and potential neurogenic sites in all the three investigated prosimian primates. However there were variations in the pattern, rate, morphology and the density of immunopositive cells in the three species.

## 5.1 Established active neurogenic sites

### Subventricular zone, and Olfactory bulb

According to reports (Amrein *et al.*, 2007; von Bohlen *et al.*, 2007; Ihunwo and Pillay, 2007; Kam, 2008; Ihunwo, 2011; Kempermann, 2012; Chawana *et al.*, 2013; Patzke *et al.*, 2015) the active neurogenic sites in adult mammalian brains are the Subventricular zone (SVZ) of the lateral ventricle, olfactory bulb and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus. The cells from the SVZ migrate through the rostral migratory stream to the olfactory bulb. Two type of neurons are reported to be generated during adulthood in the olfactory bulb and most of these neurons develop into granule cells with a small percentage becoming interneurons (Kempermann *et al.*, 2004a). Despite the marked reduction of olfactory bulb in these three species, the SVZ was mitotically active and provided neuroblasts to the olfactory bulb as revealed by DCX and Ki-67 positive cells. Immature DCX positive neurons were identified in the SVZ of the lateral ventricle in all the three prosimian primates.

These immature neurons were migrating around the caudate nucleus forming migratory chains to the olfactory bulb. In the olfactory bulb the cells were more concentrated in the granular cell layer, and migrated radially to the outer layers of the olfactory bulb. The density of DCX positive cells was greater in the Lemur as the layer of the SVZ was thicker and the intensity of staining of neural processes was increased compared to the other two species. The results of this study were in agreement with the results observed in most of the primates studies such as in Old world monkeys (*Macaca Mulata and Macaca fascicularis*) (Kornack and Rakic, 2001; Pencea *et al.*, 2001; Gil-Perotin *et al.*, 2009; and Wang *et al.*, 2011) and New world monkeys (Bernier and Parent, 1998 a, b; Gould *et al.*, 1998; Marlatta *et al.*, 2011).

The anatomical arrangement of the adult forebrain subventricular zone and its extensions is said to be dependent on the species and the conformation of its cerebral ventricle (Bonfanti and Peretto, 2007). In rodents, the anterior region of the SVZ produced neuroblasts that migrate in chains towards the olfactory bulb and a similar pattern of neuron distribution was observed in squirrel monkeys *(Saimiri Sciureus)* by Bedard and colleagues (2002). However in the squirrel monkeys, the SVZ had two major enlargements of which one was located at the rostral tip of the lateral ventricle beneath the rostrum of corpus callosum and another one bordering the caudal tip of the caudate nucleus (Bedard *et al.*, 2002). The authors reported that the anterior portion was more densely populated, much thicker and more immunoreactive than the posterior part. In these investigated three prosimian primates, the SVZ also had two major enlargements and both the anterior and the posterior parts of the SVZ were thicker, densely populated and highly immunoreactive. In addition some of neurogenic cells were observed spreading to the adjacent structures such as the corpus callosum and the caudate nucleus.

Ki-67 is known to be a reliable marker of mitosis because it is expressed in dividing cells for the entire duration of their mitotic process (Kee *et al.,* 2002). Studies thus far reported that Ki-67 is expressed during mitosis in all mammalian species from rodents (Tanapat *et al.*, 1999) to humans (Eriksson *et al.*, 1998). In this study, Ki-67 proved to be a reliable proliferative marker as immunopositive cells were observed in the active neurogenic sites, namely the Subventricular zone (SVZ), the rostral migratory stream (RMS) and the olfactory bulb (OB). However the density of Ki- 67 cells was greater in the Potto compared to the Lemur and the Galago.

**The dentate gyrus of hippocampus**

The mammalian dentate gyrus is a specialized brain region in which neurogenesis continues into adulthood regardless of the developmental pattern of the animal (Gould *et al.,* 1997). Adult hippocampal neurogenesis has been reported to be a common feature across many mammalian species, with exception in the aquatic mammals such as the cetaceans (whales, dolphins and porpoises) where the lack hippocampal neurogenesis was reported (Patzke *et al.,* 2015). More primate studies such as those in humans (Erikson *et al.,* 1998; Ernst *et al.*, 2014); in treeshrews (Gould *et al.*, 1997); in Marmoset (New World Monkeys) (Gould *et al.*, 1998); and in Macaques (Old World Monkeys) (Gould *et al.,* 1999a; Kornack and Rakic, 1999).

In the investigated prosimian primates namely the Lemur, Potto and the Galago the results demonstrated the production of new granule cell neurons in the dentate gyrus, and were observed in the hilus and the subgranular zone. These neurons were migrating to the granule cell layer, with some of the cells especially in the dentate gyrus of the Lemur extending their axons and dendrites to the molecular layer. The density and the intensity of staining of DCX immunopositive cells was higher in the Lemur compared to the other two species, while the

intensity of staining and density of Ki-67 immunopositive cells in the dentate gyrus of Potto was higher compared to the Lemur and the Galago.

**The rostral migratory stream and temporal migratory stream**

The rostral migratory stream (RMS) is the major pathway by which progenitor cells migrate from the subventricular zone (SVZ) to the olfactory bulb (OB). This pathway of cells is present in the rodents (Lois and Alvarez-Buylla, 1994; Craig *et al.*, 1999), rabbits (Fasolo *et al.*, 2002; Ponti *et al.*, 2006; Bonfanti and Ponti, 2008) squirrel monkeys (Bedard *et al.*, 2002), rhesus monkeys (Pencea *et al.*, 2001) and Megachiropterans (Chawana *et al.*, 2013) brain. In rodents neuroblasts within the rostral migratory stream (RMS) migrate over one another, giving rise to the notion of 'chain migration' (Craig *et al.*, 1999; Lois and Alvarez-Buylla, 1994).

Prosimian primates extensively use their olfactory system in rearing, food foraging and scent marking behaviour for the purpose of mating and territorial marking which explains the robustness of neurogenesis observed along the rostral migratory stream and olfactory bulb. In the prosimian primates investigated, even though the size of olfactory bulb seems reduced with the increasing brain size, the cell density of DCX immunopositive cells was greater in the Lemur than in the other two species. Like in rodents and other non-human primates, this study confirms that new neurons are produced in the rostral migratory stream and the olfactory bulb of the three prosimian primates studied.

In this study the RMS was composed of a dorsal and a rostral limb which continues to merge in the OB. The dorsal limb was running ventro-caudally from the SVZ along the anterior boundary of the caudate nucleus to the sub-cortical white matter of the anterior olfactory cortex and the rostral limb was extending ventro-rostrally into the olfactory tract to the OB.

In all the three species DCX positive cells were observed in the temporal migratory stream. These positive cells were observed in the temporal horn of the lateral ventricle and were running towards the second layer of piriform cortex and the amygdala. This migratory pathway was observed other mammalian studies such as in squirrel monkeys (Bedard *et al.*, 2002), in adult rabbits (Bonfanti and Ponti, 2008) and Megachiropterans (Chawana *et al.*, 2013). A substantial amount of Ki-67 proliferating cells were observed in the amygdala and along the temporal migratory stream (TMS) of Potto, with none observed in the amygdala and along temporal migratory stream (TMS) of the Galago. However in the Lemur positive Ki-67 cells were observed in the amygdala and temporal migratory stream (TMS) with none observed in the piriform cortex.

## 5.2 Potential neurogenic sites

Most mammalian studies reported newly generated neurons in various areas of the brain such as in the neocortex (Altman, 1963;Huang *et al.*, 1998; Gould *et al.*, 1999; Gould *et al.*, 2001; Bernier *et al.*, 2002; Fowler *et al.*, 2002; Dayer *et al.*, 2005), the olfactory bulbs (Fukushima *et al.*, 2002; Gritti *et al.*, 2002; Bedard & Parent, 2004; Liu & Martin, 2003; So *et al.*, 2008; Vergano-Vera *et al.*, 2009; Giachino &Taylor, 2009), the piriform cortex (Bernier *et al.*, 2002; Pekcec *et al.*, 2006), the amygdala (Bernier *et al.*, 2002; Fowler *et al.*, 2002), the striatum (Bedard *et al.*, 2002a; 2006) .In this study both immature and maturing neurons were found in the potential neurogenic sites such as the neocortex, cerebellum, amygdala, striatum, and piriform cortex.

**Cerebral cortex**

Earlier studies on neocortical neurogenesis established that the new neurons originated in the SVZ and migrate to the neocortex where they fully mature (Gould *et al.*, 1999b, 2001; Pencea *et al.*, 2001; Bernier *et al.*, 2002; Dayer *et al.*, 2005; Shapiro *et al.*, 2009). However the

existence of adult neurogenesis in the neocortex has been a controversial issue and it is not as wildly accepted due to studies that reported negative results (Kornack and Rakic, 2001; Rakic 2002; Ehninger and Kempermann, 2003; Koketsu *et al.*, 2003). More so studies by Bhardwaj and colleagues (2006) and Spalding and colleagues (2005), through 14 C dating also reported not finding neurogenesis in the cortex of adult humans. They however reported neocortical neurogenesis to be limited to the infant stage.

More recent studies (Vessal and Darian – Smith, 2010; Klempin *et al.*, 2011; Bloch *et al.*, 2011; Marlatt , 201 *et al.*, Bonfanti and Nacher, 2012; Yang *et al.*, 2015) confirmed that cells with morphological and electrophysiological properties of immature neurons were present in the layer II with some in Layer III of the mammalian cerebral cortex. Similarly in the current study clusters of DCX positive cells were expressed in layer II of the cerebral cortex and their dendrites were directed towards layer I. These cells seemed be migrating from the subventricular zone through other migratory pathways mentioned in this study. Cells expressing Ki-67 have been observed in adult rats (Dayer *et al.*, 2005) and Megachiropterans (Chawana *et al.*, 2013). In contrast to this, No positive Ki-67 immunopositive cells were observed in the cerebral cortex in all the three species in the current study.

**The Piriform cortex and Amygdala**

The piriform cortex of rodents was reported to express immature neuronal markers such as Polysiliated neural cell adhesion molecule (PSA-NCAM) (Bonfanti *et al.*, 1992; Nacher *et al.*, 2002a) and DCX (Nacher *et al.*, 2002b). More studies reported the presence of immunopositive cells in the second layer of the piriform cortex (Bernier *et al.*, 2002; Pekcec *et al.*, 2006; Shapiro *et al.*,2007) and that the origin of these cells is periventricular (Nacher and Bonfanti, 2012). However a lot of controversy sparked as to whether the neurons found are newly generated during adulthood or they are of embryonic origin. Some studies reported

that that some of the cells in the piriform cortex layer II have been generated in adulthood (Bernier *et al.,* 2002; Pekcec *et al.,* 2006; Shapiro *et al.,* 2007) while studies like that of Gomez- Climent and colleagues (2008) provided evidence that the cells are of embryonic origin because they appeared to be in a prolonged immature state.

In all the investigated prosimian primates cells expressing DCX were observed in the piriform cortex and amygdala with more cells migrating from the SVZ of the temporal horn. The migration of these DCX positive cells along the temporal migratory stream (TMS) was observed to end in the second layer of the piriform cortex (Figure 4.6). In the Amygdala DCX positive cells were observed inside and along its borders and most of the cells had their dendrites directed in different directions. Majority of the cells expressed in this area had an immature neuron appearance with few of the cells having a mature nature. In the three species the Galago had no Ki-67 positive cells expressed in the piriform cortex and amygdala. Few Ki-67 proliferating cells were spreading in the deep layers of the piriform cortex in Potto.

**Striatum**

The occurrence of adult neurogenesis in the striatum has been investigated in several mammalian species like in rats (Dayer *et al.*, 2005); mice (Shapiro *et al.*, 2009); Rabbits (Luzzati *et al.*, 2006); Monkeys (Bedard *et al.*, 2002; 2006) and Humans (Ernst *et al.*, 2014).The striatal newly generated neurons are suggested to be originating from the adjacent subventricular zone (SVZ) (Bonfanti and Peretto, 2011).

In the current study positive DCX cells were densely distributed in the caudate nucleus with some spreading to the putamen and nucleus accumbens and few observed spreading to the claustrum and septum. Similarly findings by Bedard and colleagues (2002a; b) established that new neurons in the adult squirrel monkeys are produced throughout adult life in the striatum and develop a mature neuronal phenotype. Dayer and colleagues (2005) reported BrdU and DCX immunoreactive cells in the striatum of rats and these cells were seen to be migrating from the SVZ, however these cells were reported to be microglial in nature and this pathway was also reported to occur due to pathological conditions such as ischemia. The striatum is reported to enlarge in parallel with the cerebral cortex and is well developed in higher animals, thus the higher animals are expected to have more pronounced striatal neurogenesis. Ernst and colleagues (2014) reported that new neurons are generated in the striatum of the human brain and that these neurons migrate from the SVZ, and that positive cells were greater in the caudate nucleus than in the putamen, claustrum, internal capsule and nucleus.

Similar pattern of cell distribution was observed in this study however no cells were observed spreading to the internal capsule. Similarly to the squirrel monkeys (Berdard *et al.*, 2002), new neurons in the striatum are added postnatally because the Ki-67 immuno- positive cells were often found in smaller clusters of 2 or 4 cells with more clusters in the Potto compared to the other two species, immature neurons were migrating to the striatum and develop a mature neuronal phenotype there.

**Cerebellum**

In the mammalian cerebellum a huge population of granule cells is established during the period in which the animal is interacting with the external environment in the process called the granule cell agenesis (Bonfati and Peretto, 2011). In this study the immature neuron marker DCX was found expressed in two of the studied species (Lemur and Galago) with none observed in one species (Potto) .The DCX positive cells observed in the cerebellum of the Lemur were mostly bipolar with some unipolar and few multipolarized, while in the Galago they were mostly unipolar. These cells were extending their apical dendrites to the molecular layer of the cerebellum with some cells in the Lemur reaching the deep part of the molecular layer with their apical dendrites. Also some of the multipolarized cells were spreading their dendrites in different cell.

A confirmation of this was in the study by Ponti and colleagues (2008) who observed bipolar, intermediate and polarized neurons were also observed in the adult rabbits that expressed the DCX and PSA-NCAM. No Ki-67 positive cells were observed in the cerebellum of all the species. These results were in contrast with the findings by Chawana and Colleagues (2013) who reported scantly distributed Ki-67 positive cells in the cerebellum of all the Megachiropterian species but no DCX positive cells.

Hence the results of this study confirmed that indeed cerebellar neurogenesis vary according to species, the environmental exposure and the period of protracted neurogenesis. The occurrence bipolar, intermediate and polarized neurons observed in these species and the absence of Ki- 67 proliferating cells, do confirm that no new neurons develop in the cerebellum but neurons migrate to the cerebellum and develop a mature nature with time.

### 5.3 Categorizing the morphology DCX positive cells in the DG

In all the investigated species, DCX positive cells were observed in the subgranular zone of the dentate gyrus. Majority of the DCX positive cells according to Plumpe and colleagues (2008) classification, were in post mitotic stage (stage E to F) and very few in the intermediate stage (C to D). However the interspecies comparison of DCX positive cells categorization, revealed that the majority of the cells classified were under category F in the Lemur catta while Category E cells were most abundant in the Galago and Potto.

Several studies reported that stress has an effect in adult hippocampal neurogenesis however this depends on the time the animal has been exposed to a stressful condition (Watanabe *et al.*, 1992; Tanapat *et al.*, 2000; Czeh, *et al.*, 2007; Parihar *et al.*, 2009; Lyons *et al.*, 2010; Marlatt *et al.*, 2011). Watanabe and colleagues (1992) and Czeh and colleagues (2007) demonstrated that chronic stress alters dendritic morphology in the hippocampus. Chronic stress has been reported to result in persistent inhibition of granule cell production and change the structure of the DG (Gould *et al.*, 1999). Therefore the longer the time the animal spend in a stressful condition, the greater the rate of inhibition of granule cells. Other studies in monkeys, by Marlatt and colleagues (2011) investigated the effects of psychosocial stress in common marmoset monkeys after exposure for two weeks and found that it failed to alter the number of BrdU positive and DCX positive cells both in the hippocampus and the amygdala.

As much as chronic stress plays a role in reducing the neurogenesis, the short period of stress has minimum effect thus in this study we could assume that capture stress have not played a role in reducing the amount of newly generated neurons because the animals that were caught in the wild such as Galago and Potto, were euthanized within 8 hours of being captured. The scantly distributed DCX positive cells Galago and Potto could not be due to capture stress

thus other factors might have contributed to this. Also environmental enrichment of the animal might have not contributed much to affect the rate and intensity of the neurogenesis as both animals that were caught in the wild (Potto and Galago) had a similar morphology of DCX positive cells with most of the cells falling under category E and few in category F compared to the Lemur which was caught in the zoo, most of the cells were in category F (Cell showing mature appearance with dendrite reaching the molecular layer and showing dense branching with few branches near the soma or within the granule cell layer) with few in category E (Cell showing mature appearance with one dendrite penetrating the molecular layer and showing sparse dendritic branching) (Plumpe *et al.*, 2006).

Also supporting this notion that the environment played no role, was the results on Ki-67 proliferative marker. The Potto had the highest density and rate of Ki-67 proliferative cells compared to the other two species. Occasionally some cells had their dendrites running horizontally to the subgranular zone layer (SGZ) of the dentate gyrus (DG) with some cells with unipolar, bipolar, no processes and with short processes observed in all the species. In the Megachiropterans no variations were observed in the DCX cells in all the 8 species studied, majority of the cells had bipolar processes that extended into the molecular layer (Chawana *et al.*, 2013).

### 5.4 Proliferating cell number of Ki-67

Potto and the Galago species used were caught in the wild, therefore one would expect that the rate of proliferating cells will be lower in these species due to exposure to predator stress during capturing. However the Potto showed no effect of predator stress because they had the highest rate of proliferative cells compared to the zoo kept Lemur which are used to being exposed to predators (zoo visitors and workers). When comparing the rate of cell

proliferation between the species, Potto had 1.9 times higher rate of proliferative cells than the Lemur and 4.8 times higher than the Galago. Lemur had about 2.5 times higher rate of proliferative cells than the Galago.

The statistical analysis (Student t-test) confirmed that the rate of Ki-67 proliferating cells was significantly higher in Potto compared to the Lemur and the Galago ($p \leq 0.05$) and the rate of proliferating Ki-67 cells between the Lemur and Galago was not significantly different ($p \geq 0.05$). The above results proved that other factors might have contributed to such variation. These results were in contrast with the study by Tanapat and colleagues (1999) who observed no difference in the rate of Ki-67 positive cells between male and female rats. However the variability in the rate of proliferating cells in this study could not be attributed to the age of the animal even though the exact chronological ages of the animals was not known. The weight of the animal was used to determine the age. For instance literature recorded the weight of the adult Potto to ranging from 850 g to 1600 g; Lemur ranging from 1150 g to 2045 g and Galago ranging from 44g to 74g therefore the species used were all adults because their weight was 1400g for both Potto species, 74 g for the Galago and 1750g for the Lemur.

Chawana and colleagues (2013) observed that for every doubling in brain mass the number of proliferating cells is increased 1.8 times, however in this study such trend was not observed because the brain mass of the Potto was almost half that of the Lemur. A statistical analysis of multiple regressions was run in order to confirm if there is any correlation in the brain mass and the number of Ki-67 cells. The results on regression analysis suggested that only 13% of variation in Ki67 count was explained by brain Mass (R-squared=0.1288) and that if brain mass is increased by 1 unit (1gr), the overall cell count is expected to increase by 41

cell counts (coefficient=40.63, Std. Error =52.84). However, this has been statistically not significant (p - value=0.485), thus it is strongly suggested that an increase in the sample size may give significant results.

## CHAPTER 6: CONCLUSION

This study provided evidence that adult neurogenesis is present in the active sites and potential sites in the brains of the investigated prosimian primates, namely the *Lemur catta*, *Perodictus potto* and the *Demidoff dwarf galago*. The DCX and Ki-67 immunopositive cells were observed in the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus. The identified potential sites were the cerebellum, striatum, cerebral cortex, amygdala, and the piriform cortex.

The pattern of neurogenesis was similar in all the species, as all species showed to have cells migrating from the SVZ of the lateral ventricles along the rostral migratory stream to the olfactory bulb. These primates also had a secondary pathway, temporal migratory stream. In this pathway, the DCX positive cells were migrating from the temporal horn of the lateral ventricle to the amygdala and the piriform cortex in all the species. In the striatum, the DCX immunopositive cells were scantly distributed in all the species.

Despite the difference in cell density and the rate of cell proliferation in this study, there was evidence of neuronal pathways such as the rostral migratory stream (RMS) which is prominent in rodents, the temporal migratory stream (TMS) which prominent in non-human primates and Megachiropterans and the striatal neurogenesis which is recently confirmed in human primates.

Despite all the contradictory studies on neocortical neurogenesis, it was evident in these prosimian primates. Abundant immature neurons and few mature DCX immunopositive cells were present in the potential sites such as the cerebellum, amygdala, piriform cortex, striatum, and the neocortex. In conclusion the prosimian primates revealed patterns of neurogenesis that are similar to that observed in other primates.

## RESEARCH LIMITATION

Inability to determine the effect of hormonal influence as the gender of most of the species used was unknown.

## FUTURE STUDY RECOMMENDATIONS

1. Double immunostain labelling in order to explain and answer questions about why some brain areas had neurogenesis with one marker and no neurogenesis with the other marker.
2. Stereological quantitative studies such as optical fractionator to estimate the granule cell number of the dentate gyrus is recommended.

**APPENDICES**

**Ethics clearance certificates**

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